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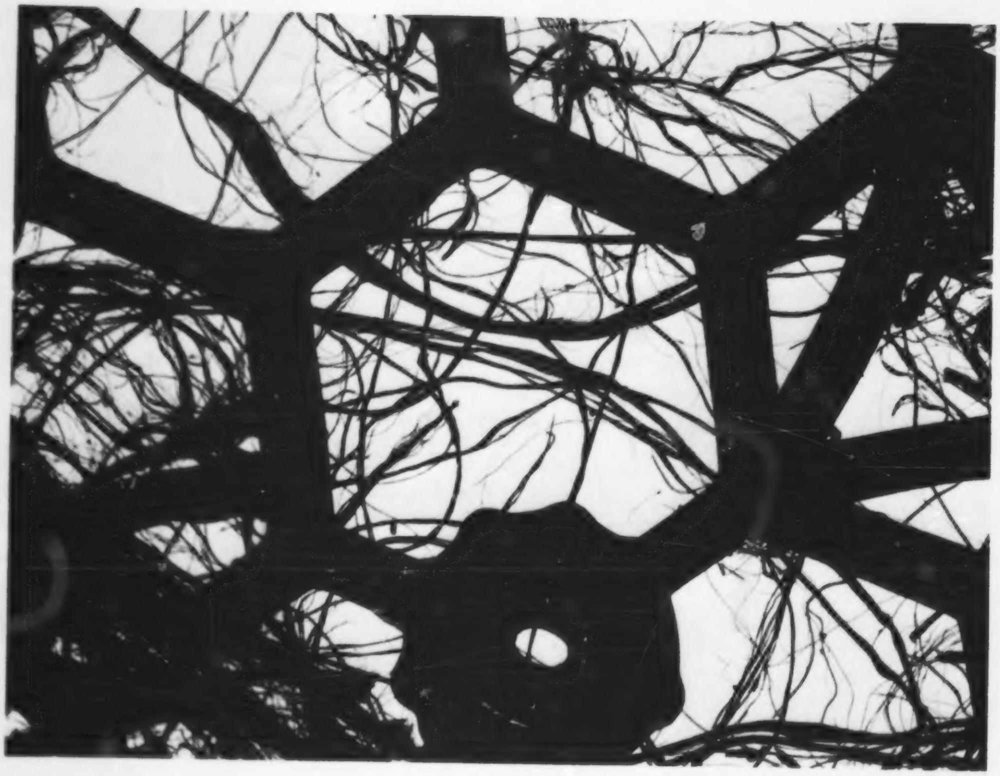
ASBESTOS STANDARDS:
Materials and Analytical Methods



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This publication contains the invited papers which were presented at a workshop on asbestos standards jointly sponsored by the Environmental Protection Agency and the National Bureau of Standards and held on October 1-3, 1980. The workshop was divided into five sections: (1) Bulk Materials for Preparation of Asbestos Standards....This section includes descriptions of natural and synthetic materials which have a potential use as standards for asbestos analysis. In addition, it also includes a description of the NBS Standard Reference Materials Program. (2) Standards Preparation.... The electron-microscopy preparation procedures for standards mimicking airborne and waterborne asbestos samples are described. (3) Asbestos Analysis for Standards Certification....This section describes analytical procedures and problems associated with the intra- and interlaboratory analyses of asbestos standards. (4) Error Analysis and Statistics....This section describes the statistical considerations which are involved in asbestos standards preparation and analysis. (5) EPA Provisional Method....Current developments and selected problems with the EPA Provisional Method for Electron Microscope Measurement of Airborne Asbestos Concentrations are discussed.

The papers include general reviews on each of the subjects as well as specific papers detailing current research efforts.

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John Small and Eric Steel

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FOREWORD

The Center for Analytical Chemistry of the National Measurement Laboratory, National Bureau of Standards, seeks to develop new techniques of chemical analysis and to constantly improve existing analysis methods. Part of the mission of NBS is to disseminate knowledge in the scientific and technical community. To aid in achieving this objective, the Center for Analytical Chemistry has sponsored a series of workshops on various topics in analytical chemistry. The workshop topics are chosen to fulfill current needs for detailed discussions on well-defined subjects in a wide variety of specialized areas of interest. The objective of these workshops is to bring together specialists from throughout the world to concentrate intensively on a particular subject in order to advance the state-of-the-art. It is often very difficult to achieve this goal at large international meetings where the size and diversity of topics presented often limit detailed discussion of specialist subjects. Past topics of these workshops and the published proceedings include: Quantitative Electron Probe Microanalysis (NBS Special Publication 298), Aerosol Measurements (NBS Special Publication 412), Secondary Ion Mass Spectrometry (NBS Special Publication 427), Use of Monte Carlo Calculations in Electron Probe Microanalysis and Scanning Electron Microscopy (NBS Special Publication 460), Characterization of Particles (NBS Special Publication 533), and Energy Dispersive X-ray Spectrometry (NBS Special Publication 604). These proceedings are available from the Superintendent of Documents, Government Printing Office, Washington, D. C. 20402. Further information on the workshops can be obtained by writing to the Center Office, Center for Analytical Chemistry, National Bureau of Standards, Washington, D. C. 20234.

This volume contains the proceedings of a Workshop on Asbestos Standards. The three-day meeting involved participants from the United States and Canada. The workshop format consisted of invited talks and contributed papers on each topic followed by extensive discussions.

There is an important need in the area of asbestos analysis for physical standards as well as standard analytical methods. This publication provides a detailed view of the state-of-the-art in asbestos standardization and analysis. The papers should be of interest to workers involved in programs associated with all aspects of asbestos measurement.

PREFACE

Asbestos has been an important manufacturing and industrial product since the beginning of the industrial revolution resulting in its use in a variety of goods and materials. It was not until the mid 1900s that asbestos was recognized as a health hazard and by that time its widespread use had created a detectable global background level. As a result of its potential adverse health effects, the regulation and monitoring of asbestos has increased dramatically during the past several years.

In 1976, the National Bureau of Standards (NBS) at the request of the Occupational Safety and Health Administration (OSHA) analyzed 80 industrial talc samples for asbestos. This analysis marked the initial involvement of the Center for Analytical Chemistry at NBS in the analysis for and characterization of asbestos.

As a direct result of this program, NBS and OSHA jointly sponsored a workshop on asbestos. The workshop was purposefully broad in scope, enabling NBS and OSHA to evaluate information concerning the current state-of-the-art in asbestos definitions and measurement methods. The proceedings of the workshop were published as NBS Special Publication 506.

Since this initial work, the emphasis of NBS involvement with asbestos has appropriately shifted from routine analysis to a joint program with the Environmental Protection Agency for the development of quality control specimens, reference standards, and refinement of analysis methodology. As part of this program, a joint NBS-EPA workshop on Asbestos Standards was held in October 1980. This workshop was designed to bring together representatives from private and governmental organizations which are actively involved in asbestos standards preparation and development of analysis methods.

The editors gratefully acknowledge the excellent work of Joy Shoemaker and the members of the Text-Editing Facility of the Center for Analytical Chemistry in preparing this manuscript.

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E. B. Steel

Gas & Particulate Science Division
Center for Analytical Chemistry

ABSTRACT

This publication contains the invited papers which were presented at a workshop on asbestos standards jointly sponsored by the Environmental Protection Agency and the National Bureau of Standards and held on October 1-3, 1980. The workshop was divided into five sections: (1) Bulk Materials for Preparation of Asbestos Standards....This section includes descriptions of natural and synthetic materials which have a potential use as standards for asbestos analysis. In addition, it also includes a description of the NBS Standard Reference Materials Program. (2) Standards Preparation....The electron-microscopy preparation procedures for standards mimicking airborne and waterborne asbestos samples are described. (3) Asbestos Analysis for Standards Certification.... This section describes analytical procedures and problems associated with the intra- and interlaboratory analyses of asbestos standards. (4) Error Analysis and Statistics....This section describes the statistical considerations which are involved in asbestos standards preparation and analysis. (5) EPA Provisional Method....Current developments and selected problems with the EPA Provisional Method for Electron Microscope Measurement of Airborne Asbestos Concentrations are discussed.

The papers include general reviews on each of the subjects as well as specific papers detailing current research efforts.

Key Words: Asbestos standards; asbestos statistics; electron microscopy; fibrous minerals.

TABLE OF CONTENTS

PAGE

FOREWORD . . . iii
PREFACE . . . iv
ABSTRACT . . . v

INTRODUCTION

QUALITY ASSURANCE FOR AIRBORNE ASBESTOS MEASUREMENTS . . . 1
Michael E. Beard

SESSION I. BULK MATERIALS FOR PREPARATION OF ASBESTOS STANDARDS

ASBESTOS REFERENCE MATERIALS: SOURCES AND CHARACTERIZATION . . . 5
Jean L. Graf, Ronald G. Draftz, and Janet C. Haartz

GLASS AS A MATERIAL FOR ASBESTOS STANDARDS . . . 21
David J. Cronin, Douglas H. Blackburn, and Wolfgang K. Haller

THE NBS PROGRAM FOR STANDARD REFERENCE MATERIALS . . . 29
R. Keith Kirby

IDENTIFICATION AND QUANTIFICATION OF ASBESTOS IN CONSTRUCTION MATERIALS USING POLARIZED LIGHT MICROSCOPY: THE NEED FOR STANDARDS . . . 34
R. L. Virta, K. B. Shedd, and W. J. Campbell

PREPARATION OF ASBESTOS "STANDARDS" FOR METHODS VERIFICATION AND LABORATORY EVALUATION . . . 44
D. E. Lentzen, E. P. Brantly, K. W. Gold, and L. E. Myers

SESSION II. STANDARDS PREPARATION

SAMPLE PREPARATION FOR QUANTITATIVE ELECTRON MICROSCOPE ANALYSIS OF ASBESTOS FIBER CONCENTRATIONS IN AIR . . . 53
Philip M. Cook and David R. Marklund

DEVELOPMENT OF AN ASBESTOS REFERENCE SUSPENSION . . . 68
Richard S. Feldman

PREPARATION OF AIRBORNE ASBESTOS STANDARDS . . . 77
David R. Jones and George Yamate

CONCENTRATION AND SEPARATION OF CHRYSOTILE BY TWO-PHASE LIQUID SEPARATION . . . 85
Carl W. Melton, Sandra J. Anderson, Carolyn F. Dye,
W. Eugene Chase, and Charles H. Anderson

SESSION III. ASBESTOS ANALYSIS FOR STANDARD CERTIFICATION

ANALYTICAL PROCEDURES AND STANDARDIZATION FOR ASBESTOS FIBER COUNTING IN AIR, WATER, AND SOLID SAMPLES . . . 91
Eric J. Chatfield

DEVELOPMENT OF A RAPID SURVEY TECHNIQUE FOR THE DETECTION OF ASBESTOS FIBERS . . . 108
Peter Riis and Eric J. Chatfield

Table of Contents Continued PAGE

INTERLABORATORY MEASUREMENTS OF THE CHRYSOTILE ASBESTOS FIBER AND MASS CONCENTRATIONS IN WATER SAMPLES. 121
K. S. Chopra, D. Beaman, and P. Cook

CONSIDERATIONS IN THE ANALYSIS AND DEFINITION OF ASBESTOS USING ELECTRON MICROSCOPY 132
R. J. Lee, J. F. Kelly, and J. S. Walker

COMMENTS ON THE ACHIEVABILITY OF A VALID ASBESTOS STANDARD FOR TEM COUNTING. . . 138
I. M. Stewart

SESSION IV. ERROR ANALYSIS AND STATISTICS

MEMBRANE FILTER METHOD: STATISTICAL CONSIDERATIONS. 145
Gerald R. Chase

SIMULATION OF THE EPA PROVISIONAL METHOD FOR AIRBORNE ASBESTOS CONCENTRATIONS 154
Terence Fitz-Simons and Michael E. Beard

ANALYTICAL ERRORS IN ASBESTOS ANALYSIS BY ANALYTICAL ELECTRON MICROSCOPY 162
Eric B. Steel, John A. Small, and Patrick Sheridan

STATISTICAL CONSIDERATIONS IN THE PREPARATION OF CHRYSOTILE FILTER REFERENCE MATERIALS: FILTER HOMOGENEITY 169
Stephan Leigh, Eric Steel, John Small, Patrick Sheridan, and James Filliben

SESSION V. EPA PROVISIONAL METHOD

REFINEMENTS IN THE EPA PROVISIONAL METHODOLOGY 183
George Yamate and Michael E. Beard

FIBER IDENTIFICATION AND BLANK CONTAMINATION PROBLEMS IN THE EPA PROVISIONAL METHOD FOR ASBESTOS ANALYSIS 190
Steven Ring and Robert J. Suchanek

MSHA STANDARD METHOD FOR FIBER IDENTIFICATION BY ELECTRON MICROSCOPY 207
R. L. Clark

LIST OF ATTENDEES 211

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QUALITY ASSURANCE FOR AIRBORNE ASBESTOS MEASUREMENTS

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Abstract

An EPA program designed to provide quality assurance for measurement of airborne asbestos fibers is outlined. The program provides for the development of (1) a standardized measurement protocol and (2) a characterized reference material suitable for performance evaluation. A provisional methodology manual (EPA Report 600/2-77-178) describing transmission electron microscopic identification of asbestos fibers is being evaluated under contract. Critical subroutines involving sampling, sample preparation, and analysis are being investigated and the resulting information will be used to optimize the current protocol. The resulting protocol will be subjected to a multilaboratory collaborative test designed to determine precision and accuracy of the method. A concurrent EPA-NBS agreement is designed to produce reference materials for use in performance evaluation of electron microscopic analysis of asbestos fibers. The materials will be in the form of prepared grid specimens and coated sample filters. These standards will allow identification of variabilities due to sample preparation and counting. The program is scheduled for completion in late 1981.

1. Introduction

The hazards associated with the widespread occurrence and use of asbestos fibers have become a matter of increasing concern in recent years. The association between asbestos workers and respiratory diseases such as asbestosis and lung cancer has resulted in air quality standards for the work environment. Concern has also been raised for others who breathe ambient levels of this hazardous material. It is in the public interest that any potential health hazard due to breathing airborne asbestos fibers be assessed.

Measurement of airborne asbestos presents a unique analytical problem. These fibers may range in size from a hundred or more micrometers to submicrometer lengths and submicrometer diameters. Reliable identification of the fibers requires the use of transmission electron microscopy (TEM) which is costly, tedious, and time consuming. A recent study focused on asbestos emissions from roadways surfaced with crushed stone obtained from a quarry containing small amounts of asbestos [1][1]. Particulate samples analyzed for asbestos by several laboratories using their own analytical protocol gave results on split samples which differed by several orders of magnitude (Table 1). The need for a standardized measurement technique is obvious.

This paper reviews the Environmental Monitoring Systems Laboratory (EMSL), Research Triangle Park, North Carolina (RTP) program to standardize and provide quality assurance for airborne asbestos measurements. Detailed findings of each phase of the program will be presented by the respective investigators during this workshop. The cost of asbestos measurements and the cost and impact of controlling emissions of this hazardous material demand a reliable standardized measurement technique and a rigorous quality assurance program.

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[1] Figures in brackets indicate the literature references at the end of this paper.

Table 1. Ambient Air Asbestos Fiber Counts Measured by Eight Laboratories for Site 1 Adjacent to an Unpaved Graveled Road, Millions of Fibers/m^3 of Ambient Air.

		Laboratory Designation							
Relative Level	Sample Number	A	B	C	D	E	F	G	H
Low	1	BDL[a]	BDL	0.141	0.145	BDL	BDL	2.16	0.0334
	2	BDL	BDL	0.217	0.00739	0.00874	BDL	1.26	0.199
	3	BDL	BDL	0.160	0.264	0.00336	0.00382	0.180	1.03
Medium	11	0.00805	0.00064	0.375	0.135	0.178	0.0253	2.74	6.19
	12	0.0127	0.0262	0.297	1.48	0.510	0.0727	6.61	17.1
	13	0.00211	0.0303	0.253	1.04	0.273	0.0422	3.37	4.29
High	21	0.00521	0.0417	0.608	1.60	0.201	---	28.2	13.7
	22	0.00159	0.0333	1.15	0.339	0.100	0.160	16.8	54.9
	23	0.00845	0.0691	1.20	0.269	0.282	---	9.18	30.7

---

[a] Below detectable limits.

Data from EPA internal report: "Montgomery County Asbestos Study," October 1977.

1.1 Development of a Standardized Measurement Technique

There are several key elements which apply to the standardization of any environmental measurement technique. First, a detailed protocol for accomplishing the measurement is prepared. This protocol includes detailed descriptions of sampling, sample preparation, analysis, calibrations, and data reporting procedures. The protocol may be based on information obtained from the literature, consultation with investigators, or limited laboratory investigation. The protocol may be revised during subsequent evaluations as new information is obtained which requires modification of the procedure in order to achieve more reliable results.

Secondly, the protocol is subjected to a laboratory evaluation designed to determine the performance of the procedure under controlled conditions. Critical subroutines may be identified using multifactorial block design experiments (ruggedness tests). The optimum values for these critical parameters are then determined and the protocol is revised accordingly.

Thirdly, the protocol is subjected to various levels of field testing. This phase may include single or multilaboratory measurements. Any critical parameters identified in this phase of the study may be further evaluated in the laboratory and/or field and the information used to make revisions in the protocol.

Finally, the protocol may be subjected to a multilaboratory collaborative test designed to determine the precision, accuracy, and overall utility of the method under monitoring conditions. Performance of the procedure in monitoring networks can be measured by periodic audits using various reference standards or comparisons.

A standardization program for measurement of airborne asbestos has been in progress at EPA/RTP for the past several years. A provisional methodology manual describing the measurement of airborne asbestos was prepared under an EPA contract with IIT Research Institute in 1977 [2]. The manual was developed through consultation with several investigators and extensive laboratory evaluation [3]. In the procedure (figure 1), airborne particulate is collected on polycarbonate membrane filters. A portion of the filter is carbon coated and transferred to an electron microscope grid by means of a Jaffe wick. The grid is examined by TEM. Fibers with aspect ratios of 3:1 or greater are identified by morphology and their

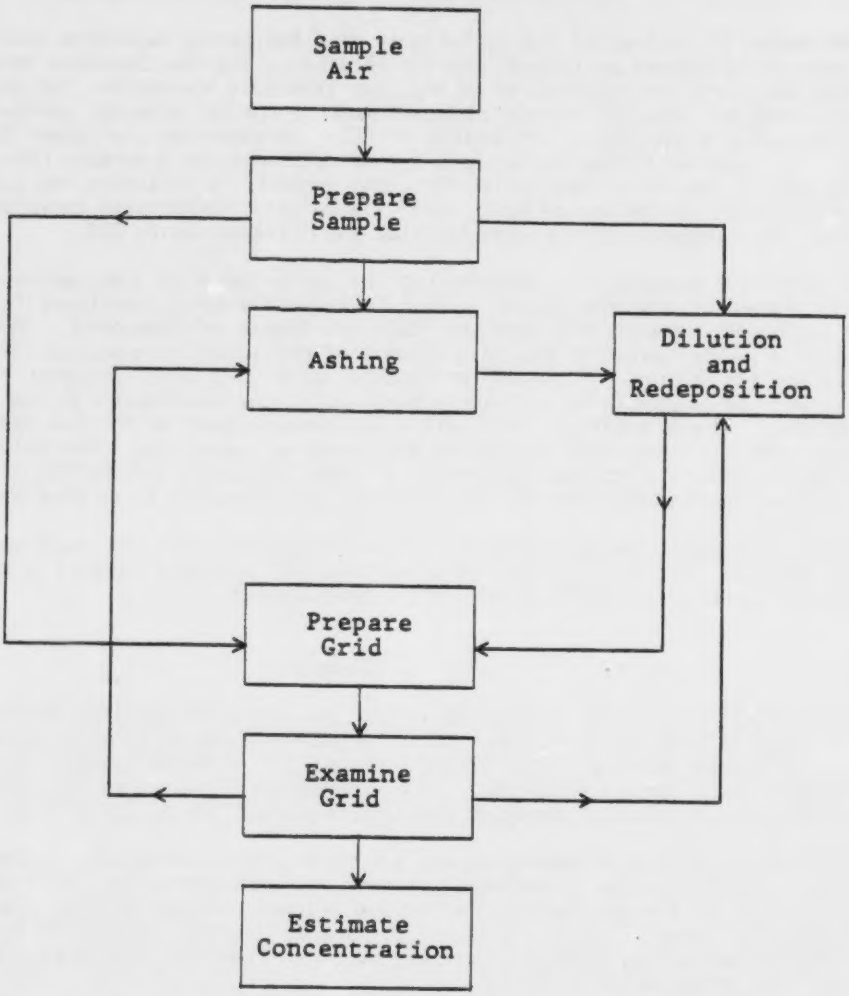

Figure 1. Flow chart of EM procedure for estimating size distribution and concentration of airborne asbestos.

crystal structure is determined by selected area electron diffraction (SAED). Chemical composition may also be determined using energy dispersive x-ray fluorescence analysis (EDXRF). Asbestos fibers are counted, their length and width determined, and a mass concentration is calculated using a density-volume relationship. Data reported includes fiber count, mass, and particle size distribution.

The method was subjected to a multilaboratory round-robin test which showed a precision of 0.48 (spread between 95 percent confidence limits to mean) for fiber number concentration and 0.40 for mass concentrations. While these results were indeed promising, some problem areas were identified in the evaluation of the method which required further study. Subroutines involving the effects of sampling with polycarbonate vs. cellulose ester filters, sampling face velocity, collection efficiency, ashing, dispersing and ultrasonic treatment of the sample, and fiber bundle counting procedures are currently under investigation by IITRI. Information obtained in the current investigation will be used to optimize the method and, if appropriate, a collaborative study will be conducted. The investigation is scheduled to be completed by 1982.

1.2 Performance Evaluation of Asbestos Measurements

One means of evaluating the performance of a monitoring technique under actual use is to provide for a comparison between results obtained using the procedure and its associated apparatus and those obtained using appropriate reference standards. For example, the flow rate of a sampler used to collect airborne particulate for asbestos analysis may be calibrated by using a gas meter, calibrated orifice and manometer, or other flow measurement device. An independent flow measurement device referenced to a primary flow measurement can then be used to obtain a comparative flow measurement. A criterion for acceptance of the user calibration can be established using established performance characteristics of the apparatus. Performance audit devices for flow are in common use by EPA.

A reference material for determining the performance of the sample preparation and analysis phases of the provisional method is currently being developed through an interagency agreement between EPA and the National Bureau of Standards. The material will consist of a filter material having a deposit of characterized asbestos fibers in an urban air particulate matrix. Electron microscope grid specimens prepared from the filter material will be available on an interim basis until the development of the filter material is completed. These materials will allow performance testing for the sample preparation technique and the fiber identification and counting techniques. The reference materials will contain several concentration levels of both chrysotile and amphibole asbestos fibers in an urban air particulate matrix. The materials are scheduled to be available by 1982.

Other standards are available to the microscopist, such as latex spheres or carbon grating replicas used to calibrate EM magnification, and gold layered grid specimens used as internal standards for diffraction pattern measurements.

2. Summary

The effort to provide quality assurance for airborne asbestos measurements will be greatly promoted with the development of (1) a standardized measurement protocol and (2) an asbestos reference material. Monitoring programs for airborne asbestos can be undertaken and reliable data can be obtained. This information can be used to assess the need for establishing any air quality standards required to protect the public health.

Progress reports from these projects are receiving review by several investigators representing federal and state government, private research institutes, and industry. Comments and suggestions on the program are invited and welcome. Please address same to the Methods Standardization Branch, Attn: Michael E. Beard, Quality Assurance Division (MD-77), Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

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[1] Montgomery County Asbestos Study, EPA internal report, U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Environmental Monitoring Division, Research Triangle Part, North Carolina 27711, October 1977.

[2] Electron Microscope Measurement of Airborne Asbestos Concentrations: A Provisional Methodology Manual, EPA Report No. 600/2-77-178, Environmental Sciences Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, Revised June 1978.

[3] Evaluating and Optimizing Electron Microscope Methods for Characterizing Airborne Asbestos, EPA Report No. 600/2-78-038, Environmental Sciences Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, June 1978.

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ASBESTOS REFERENCE MATERIALS: SOURCES AND CHARACTERIZATION

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Abstract

One of the keys to the successful development of any analytical method is the availability of well-characterized reference materials. The need for asbestos standards is especially acute since research efforts to develop standardized methods of asbestos analysis are currently in progress even though there is an absence of high purity, well-characterized asbestos reference materials. Current knowledge on sources for asbestos reference samples and the extent of their characterization are summarized along with a list of recommendations for the development of additional reference samples based on size and purity.

1. Introduction

The primary uses of standards or secondary reference materials are for instrument calibration, quality assurance, and methods development. In the case of known or potentially hazardous materials such as asbestos, a primary need for standard reference materials is also for bioassay studies.

Standard reference materials are used in instrument calibration to either check instrument response for the substance of interest or in preparation of calibration curves. Standard reference materials are essential for quality assurance programs in analytical labs: "blind" samples prepared from known standards by the laboratory quality assurance officer must be analyzed routinely, or standard addition methods must be routinely incorporated in sample batches to verify a method's accuracy and reproducibility. No development of analytical methods can logically proceed without standard reference materials.

Asbestos poses many problems to the conventional approaches of standard reference materials production, the most significant of which is that asbestos is not simply one precise material. There are six naturally occurring silicate minerals that possess the physical and chemical properties requisite for the commercial classification as asbestos. Although only three of the asbestos minerals types - chrysotile, fibrous grunerite (amosite), and crocidolite - are mined and processed in significant quantities, the other three asbestos types - fibrous habits of the amphiboles anthophyllite, tremolite, and actinolite - have either been used commercially in small quantities or can exist in non-asbestos commercial materials such as talc. Therefore, unless analysis methods are to be restricted solely to one type of asbestos, standard reference materials of all six of the asbestos types must be prepared.

The task of preparing suitable, broad application asbestos standard reference materials is further complicated by the fact that one asbestos type can occur with widely varying chemical and physical properties. In addition, the amphibole asbestos minerals also occur in nonfibrous, but elongated crystal habits that are not readily distinguished from the fibrous habit on a microscopic scale. Therefore, more than one standard reference material of each asbestos type may have to be prepared, and the non-fibrous forms of the amphibole asbestos minerals may also have to be prepared as standard reference materials to insure that developed analytical methodologies are capable of distinguishing between the asbestos and non-asbestos varieties of the mineral.

The desired properties of standard reference asbestos materials include high and documented purity; availability, or more properly, accessibility to the research and analytical communities; and availability in forms amenable to the variety of uses to which they will be put. These properties are not independent of each other - and therefore must be considered very carefully as the plans for production of asbestos standard reference materials are formulated.

2. Existing Needs for Asbestos Standard Reference Materials

Table 1 lists the most common analytical techniques currently employed in the detection and quantitation of asbestos and the types of samples that are typically analyzed by the techniques. There are, in fact, no fully developed, certified, so-called "standard" analysis methods for asbestos,[1] for the most part, because standard reference materials for methods development and methods testing have not been available.

Table 1. Current Needs for Asbestos Standard Reference Materials.

Technique	Type of Sample	Materials to be Analyzed
Polarized Light Microscopy [1][2]	bulk powders	insulations, quarried rocks, building materials
X-ray Diffraction [1]	bulk powders	insulations, quarried rocks, building materials, talcs
X-ray Diffraction [2]	thin film samples	airborne fibers, liquid-borne fibers, thin films prepared from bulk powders
Electron Microscopy [3]	thin film samples	airborne fibers, liquid-borne fibers, thin films prepared from bulk powders

Some of the existing and proposed federal regulations pertaining to asbestos have created urgent needs for asbestos standard reference materials in the analytical community by either establishing limits for asbestos concentration (in the environment or in commercial products) or by specifying that a certain analytical technique be employed. For example, the recently proposed rules of the U.S. Environmental Protection Agency [5] and the U.S. Department of Education [6] for detection and control of asbestos containing building materials in educational institutions require employment of a tentative polarized light microscopy or x-ray diffraction techniques [1] for the analyses of the suspect building materials. Since none of these tentative asbestos analysis methods has been sufficiently tested to determine precision, accuracy, reliability and interferences, those proposed rules may encounter stiff opposition, or if passed, may be nonenforceable because adequate numbers of qualified laboratories will not be available to provide the required analyses. A major stumbling block to the testing and development of the methodologies has again been a lack of asbestos reference materials. In fact, the tentative x-ray diffraction (XRD) methodologies will be useable only by a few government laboratories because both the bulk powder and thin film XRD analysis methods require the preparation of a standard calibration curve. Logically, if standard reference asbestos materials are not used by commercial analytical laboratories for instrument calibration, the XRD data produced cannot be considered reliable.

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[1] The possible exception is the NIOSH P&CAM 239, Asbestos Fibers in Air, in which fiber concentrations in the asbestos workplace environment are quantitated by a phase contrast optical microscopy counting method. It should be noted that all particles meeting the morphology and aspect ratio criterion of fibers, rather than only asbestos fibers, are counted in this technique.

[2] Figures in brackets indicate the literature references at the end of this paper.

It should be obvious from Table 1 that a wide variety of asbestos standard reference materials are required by the analytical community. In addition to standard reference materials of each of the various minerals that fall under the commercial category, asbestos, each type of asbestos should be prepared in several different forms. For example, analyses for determination of asbestos in bulk materials such as insulation by polarized light microscopy will require preparation of standards with relatively large sized fibers, while asbestos standards to be analyzed by x-ray diffraction or electron microscopy methods must be reduced to very much smaller fiber sizes. Consideration should also be given to the preparation of standard reference materials of asbestos in the various matrix types expected to be encountered in real world samples, since the sample preparation procedures for PLM, EM, and XRD analyses are as important factors as the actual instrumental analysis procedures are in ensuring accurate and reliable data. Therefore, pure asbestos bulk powder standards, asbestos plus matrix bulk powder standards, pure asbestos thin film (i.e., filter collected samples) standards, and asbestos plus matrix thin film standards are the types of standard reference materials that are needed by analytical laboratories.

3. Existing Asbestos Samples

Table 2 lists some specific and generic sources of uncharacterized, processed, and unprocessed asbestos samples. Asbestos samples of unknown purity, particle size, precise chemical composition, etc., are useful for observation of general analytical responses of asbestos materials. For example, an uncharacterized asbestos sample can be used by an electron or optical microscopist for familiarization of the analyst with physical (e.g., refractive indices, birefringence for the optical microscopist), and morphological properties. However, such uncharacterized samples are of little use in the development of quantitative analysis procedures, particularly those techniques that rely on some type of asbestos concentration vs. instrument response calibration curve in the quantitation of unknown samples.

Table 2. Sources of Uncharacterized Asbestos Materials.

- U.S. Government Agencies
    - GSA Stockpiles
    - Bureau of Mines
    - State Geological Surveys
- Public and Private Museums
    - Smithsonian
- Mineral Dealers
    - Wards Natural Science Establishment
    - Minerals Unlimited
    - Mackinaw Geological Supply
- Commercial
    - Johns-Manville
    - Union Carbide
- X-ray and Optical Microscope Analysis Supplies Dealers
    - Spex
    - Cargille
    - Somar Labs.
- Chemical Laboratories
    - Gooch Crucible Fibers

The GSA stockpiles currently maintain supplies of chrysotile, fibrous grunerite, and crocidolite asbestos. A letter of authorization is usually required from another government agency to obtain samples from the GSA stockpiles and other public institutions such as the Smithsonian Museum. Therefore, non-government laboratories seeking samples of asbestos from these government agencies must require the asbestos samples for completion of an existing government sponsored contract.

Numerous private and commercial mineral dealers operate throughout the United States. A brief search through the geology and mineralogy oriented professional journals will provide many more dealers than those in Table 2 that can be contacted for asbestos mineral specimens. Mineral dealers are especially useful as sources of the nonfibrous counterparts of the amphibole asbestos types. The three dealers listed have been especially helpful to IITRI in obtaining asbestos mineral specimens in the past; the dealers are each located in different areas of the United States that are rich in asbestos minerals or the nonfibrous counterparts.

The major U.S. asbestos industrial companies (e.g., Johns-Manville and Union Carbide) may also provide small samples of uncharacterized or partially characterized asbestos.

Supply dealers that specialize in accessories, and chemicals for x-ray and optical (polarized light) microscopy analysis techniques also sell powdered mineral sample sets that include the asbestos minerals. Caution is advised in the interpretation of analysis data from the powdered amphiboles since it cannot be determined with certainty if the minerals were of a true fibrous habit in the original, unground specimen and were therefore asbestos.

One of the more interesting sources of amphibole asbestos (usually anthophyllite) has been discovered to be the dusty back shelves of an old chemistry laboratory. The fibrous filter bed material sold for Gooch crucibles was amphibole asbestos. Although laboratory equipment supply catalogues as late as 1979 still listed amphibole asbestos for Gooch crucibles as an available item, three major suppliers recently contacted (Fisher Scientific, J. T. Baker Company, and Sargent-Welch) have indicated that they no longer sell asbestos.

Table 3 lists existing asbestos standard reference materials. It should be noted that these are "existing" materials and are not necessarily available and accessible to the general scientific community.

The UICC standard reference samples of asbestos are the most widely known, used and probably also misused asbestos standard reference materials. The materials were prepared in 1966 by the collaborative efforts of the Llandough Hospital MRC Pneumoconiosis Unit (United Kingdom) and the Pneumoconiosis Research Unit of the National Research Institute for Occupational Diseases (South Africa) [7,8]. The materials were prepared specifically to reflect the purity and size distributions of the asbestos dusts encountered by industrial workers in the mining, processing, milling, and fabrication of asbestos and asbestos-containing products. The materials were intended primarily for use in biological response studies, rather than as calibration standards for instrumental analysis and analytical methods development. Therefore, the materials are relatively impure, in terms of classical concepts of "standards".

Although the UICC standard reference samples were extensively characterized, including elemental composition determinations, a major shortcoming of the UICC asbestos samples is the lack of phase purity documentation. No data were obtained on the percentage of the actual asbestos in each sample nor were data obtained for the concentrations and types of all impurity mineral and non-mineral species present. The UICC anthophyllite is especially impure and contains up to 10 percent talc.

The size distributions reported for the UICC standard reference asbestos samples based on fiber length measurements were made by optical and electron microscopy fiber counts [8]. These data are therefore not mass size distribution data and are not easily converted into mass size data. Mass size distribution data officially supplied with the samples simply list the percentage of "respirable" particles in each sample [8]; however, since these data were obtained by sampling of dust clouds created from the samples, it is conceivable that these mass data are biased against the larger, non-respirable particles [9]. Our experience with the UICC samples suggests that they are in size ranges useful to

Table 3. Current Asbestos Standard Reference Materials.

UICC Standard Reference Samples - 500 kg each

(Prepared by National Research Institute for Occupational Diseases, South Africa)

Chrysotile - Canada
Chrysotile - Rhodesia
Crocidolite - South Africa
Anthophyllite - Finland

NIEHS Bioassay Test Materials - 440-540 kg each

(Prepared by the U.S. Bureau of Mines)

Short Fiber Chrysotile - Idria Range, California
Long Fiber Chrysotile - Quebec, Canada
Crocidolite - Kuruman Hills, South Africa
Fibrous Grunerite - Penge, South Africa
Nonfibrous Tremolite - Vanderbilt Mine, New York

NIOSH Analytical Reference Minerals - 1 kg each

(Prepared by IIT Research Institute)

Chrysotile - Idria Range, California
Crocidolite - South Africa
Fibrous Grunerite - South Africa
Fibrous Anthophyllite - Montana
Fibrous Tremolite - Rajasthani, India
Antigorite - West Chester, Pennsylvania
Riebeckite - El Paso, Colorado
Grunerite - Luce Lake, Newfoundland
Anthophyllite - Bamble, Norway
Tremolite - Pennington, South Dakota

both optical and electron microscopists for the purposes of familiarizing analysts with the physical and morphological properties of the various asbestos types. However, the size distributions are too fine to be of use as analytical standards in quantitative analyses of bulk samples by optical microscopy, and are too coarse for preparation of standard thin film samples for electron microscopy. The coarse fiber contents have also caused difficulties in sample mounting for x-ray diffraction analyses. Ideally, asbestos analytical standards for bulk sample analysis by optical microscopy would consist predominantly of fibers in the 100 µm to 1 mm size range. For electron microscopy, the asbestos analytical standard should contain fibers less than 100 µm in length, with no more than 10 percent of the sample mass contained in fibers longer than 10 µm. The asbestos analytical standard required for x-ray diffraction quantitative analysis of thin film type samples would be similar in fiber length distribution to the electron microscopy standard.

There is now some indication that supplies of the UICC standards reference asbestos samples are nearly exhausted [10]. Thus, the availability of these standard reference materials in the quantities required for use by the U. S. scientific community is doubtful.

Large stocks of a few, fine-powder, well-characterized asbestos (and non-asbestos tremolite) have been prepared for the U. S. National Institute of Environmental Health Sciences (NIEHS) under the direction of the U. S. Bureau of Mines [11]. These materials were prepared principally to study the effects of orally injested asbestos in laboratory animals.

Phase purities (i.e., percent of the desired asbestos in the total sample are estimated) [11] to be greater than 96 percent for the two types of chrysotile and 99 percent for the crocidolite. However, the XRD data reported for the "long fiber" chrysotile does suggest that the sample is less than 96 percent pure, since five other contaminant mineral phases are definitely identified, two other possible contaminant phases are tentatively identified, and one peak remains unidentified in the XRD pattern. If one simply assumes the 1 percent rule-of-thumb detection level of a component in a complex matrix by XRD, at most, the "long fiber" chrysotile can only be 95 percent pure.

The NIEHS nonfibrous tremolite and fibrous grunerite both reportedly contain other asbestos types ≃25 percent fibrous and nonfibrous serpentine in the tremolite and ≃5 percent actinolite asbestos in the fibrous grunerites. Therefore, at least these two NIEHS materials are not suitable for use as standards in development of quantitative analysis methods specific for each asbestos type.

It should be noted that the NIEHS "long"[3] and "short"[3] fiber chrysotile samples were prepared from two entirely different starting materials – i.e., chrysotile mined from different geographical and geological origins. The reported chemical composition differences between these two NIEHS chrysotile samples are not great (Table 4). However, the XRD data reported suggests that non-asbestos mineral contaminants in the "long" fiber chrysotile could be responsible for the nearly identical concentration of silicon (determined as SiO_2), magnesium (determined as MgO), and iron (determined as Fe_2O_3) in the two chrysotiles. Since XRD detection limits for the mineral phases brucite ($Mg(OH)_2$), magnetite (Fe_3O_4), and apophyllite ($KF\ Ca_4Si_8O_{20} \cdot 8H_2O$) were not discussed in the analytical data presented [11], the finding of these latter two minerals only in the "long" fiber chrysotile and the detection of five peaks for brucite in the "long" fiber chrysotile but only one brucite peak in the "short" fiber sample is interpreted to indicate these contaminant mineral phases were significantly higher in concentration in the "long" fiber chrysotile sample. Thus, the near identical compositions of the two chrysotiles is questionable. While both long fiber and short fiber chrysotile standard reference materials are urgently needed for the different types of analysis methods, it is considered more desirable to obtain the required fiber size fractions from the same starting material.

Table 4. Chemical-Instrumental Analyses of NIEHS Chrysotiles [11].

	- - - Weight-percent - - -			- - - Weight-percent - - -	
Oxides	Short Fiber	Long Fiber	Oxides	Short Fiber	Long Fiber
Al_2O_3.....	0.66	1.47	MnO.......	0.07	0.06
CaO.......	0.32	0.05	SnO.......	ND	ND
FeO.......	ND	ND	SrO.......	ND	ND
Fe_2O_3.....	2.02	2.93	Bi_2O_3.....	ND	ND
MgO.......	40.62	40.26	Cr_2O_3.....	0.17	0.06
K_2O.......	ND	0.08	NiO.......	0.17	0.06
SiO_2.......	39.77	39.90	Co_2O_3.....	0.02	ND
Na_2O.......	0.01	0.04	CO_2.......	0.78	0.51
TiO_2.......	0.03	0.04	H_2O^-......	1.54	1.17
Li_2O.......	ND	ND	H_2O^+......	12.69	12.81
Total	83.43	84.77		15.44	14.67

Grand Total: Short Fiber = 98.87 Long Fiber = 99.44

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[3] "Long" and "short" fiber chrysotile are designations given by NIEHS and the Bureau of Mines to the two different types of chrysotile samples prepared. The short-fiber material is mined as such; that is, the ore bodies from which the chrysotile is mined are practically devoid of fibers longer than 100 μm. The long-fiber chrysotile was prepared from a boarder size range commercial material that is sold to the plastics industry.

The fiber size distribution data reported for the short fiber chrysotile [11] illustrates this material's suitability for use as a standard in electron microscopy analyses and non-suitability as an optical microscopy analytical standard. Fiber size distribution data on the long fiber chrysotile indicate its limited utility as an optical microscopy analytical standard, and its unsuitability as an electron microscopy analytical standard. The usefulness of either material as an analytical standard for XRD is not readily determinable from the data presented for the samples. The remaining long fibers would likely cause difficulty in sample mounting for XRD analyses.

Fiber size distributions reported for the NIEHS crocidolite and fibrous grunerite samples [11] indicate these materials will be of limited utility as analytical reference standards for both optical and electron microscopy techniques. Mean fiber diameters and volume mean fiber lengths are reported to be 0.53 and 88 μm, respectively, for the fibrous grunerite and 0.27 and 10 μm, respectively, for the crocidolite.

Aside from the purity and particle size factors that render the NIEHS asbestos samples of limited utility as analytical standard reference materials, the general availability of these materials is somewhat doubtful. The bioassay study requirements of NIEHS will likely limit the quantities of materials that NIEHS can make available for non-bioassay studies.

Under contract to NIOSH, the IIT Research Institute (IITRI) prepared 1 kg samples of the asbestos minerals chrysotile, crocidolite, fibrous grunerite, fibrous tremolite, and fibrous anthophyllite. The nonfibrous mineral counterparts were also prepared under this contract. All materials were prepared for asbestos analytical methods development being conducted by NIOSH [12].

All the materials were prepared as comminuted powders, in particle size ranges suitable for both electron microscopy and x-ray diffraction analysis techniques. The fine particle sizes render the samples unsuitable as analytical standards for optical microscopy techniques.

The NIOSH asbestos samples all do contain small number (and mass) percentages of fibers and fiber bundles greater than 40 μm in length. In addition, the crocidolite and chrysotile samples contain significant numbers of highly tangled fiber agglomerates that are not readily dispersed. These larger fibers and especially the tangled fiber agglomerates will present many problems in the applications of these two asbestos samples as quantitative analysis standards for electron microscopy techniques.

Purities of each of the NIOSH asbestos samples were inferred to be greater than 95 percent from the analyses conducted [12]. Thus, from the purity standpoint, these materials would be useful to the general analytical community as asbestos standards. However, the relatively small quantities of materials prepared and the anticipated requirements for these materials by NIOSH virtually render the samples unavailable.

4. Selection and Preparation of Samples as Asbestos Standard Reference Materials

Based on main analysis techniques and areas for which asbestos standard reference samples are currently needed and the lack of availability of the current asbestos standard reference materials to the general analytical community, it is obvious that a well-organized effort must be put forth to prepare asbestos standard reference materials. That is, none of the existing asbestos standard reference materials is either suitable for all the analysis techniques for which they are currently required, or is available to all those analytical laboratories that need them.

In selecting the asbestos materials that are to be processed into standard reference materials, careful consideration must be given to the asbestos containing materials against which the standards will be analyzed. That is, the asbestos types and origins of the various asbestos types mined, processed, or used in the United States, should be considered in deciding the numbers and types of asbestos standard reference materials to be prepared. As a natural material, the elemental composition of a particular asbestos type is quite variable with geological origin. Since the variability in elemental composition is dependent upon both the asbestos composition itself (i.e., degree of substitution of one element by another within the asbestos mineral crystal structure), and the types and amounts of nonremovable non-asbestos contaminants present, it is important to determine if the interspecimen composition variability will have significant impact on pure asbestos quantitation analytical data obtained with various analysis protocols. If two different specimens of one asbestos type do not produce identical responses in one analytical protocol, where the response being measured is the key factor in obtaining quantitative data, then either both specimens will have to be prepared as standard reference materials, or the analytical procedure must be identified as nonuseable for asbestos quantitation. For example, if an eastern Canada chrysotile gives a different calibration curve than the Idria chrysotile in a standardized XRD procedure, then XRD can only be applied as a quantitative analysis technique for samples in which the origin of the asbestos is known and for which a calibration curve has been prepared.

It must be emphasized that the evaluation of specimens in the raw materials selection process for asbestos standard preparation include all steps of the anticipated analytical protocols, particularly sample preparation steps. In the case of an electron microscopy asbestos fiber counting analysis of samples prepared from aqueous suspensions, differences in such properties as asbestos cation solubility, hydrophility, surface water adsorptionability, etc., between the asbestos standard reference material and the asbestos in the unknown sample will prevent establishment of meaningful confidence limits in quantitative data.

Compositions of the individual amphibole asbestos types are more variable from geographical source to source than are chrysotile asbestos compositions. However, because there are so many fewer geographical sources of each amphibole asbestos type that have been and are being mined commercially than there are of chrysotile, determination of the numbers and sources of each amphibole asbestos type that should be prepared as standard reference materials is far less complicated. Consider that over the past three decades, more than 35 different countries, including the U.S. itself, are reported to have been or are currently producing chrysotile at a level of at least 1000 short tons annually [13]. In addition, within a country, chrysotile may be mined from several distinctly different geological occurrences. Countries producing exportable quantities of all amphibole asbestos types number less than a dozen. Therefore, it is probable that only one standard reference material (but in several forms) of each amphibole asbestos type need be prepared for the various requirements of the analytical community, while multiple source derived chrysotile asbestos standard reference materials will probably have to be prepared.

Figures 1 through 5 illustrate the wide variability in appearance that chrysotile asbestos assumes. Table 5 lists the chemical compositions of some of these as well as other chrysotile samples. The chemical composition data would seem to indicate very little difference between chrysotile samples collected from various geographic locations. However, as mentioned previously, chemical compositional data reflect the composition of the actual chrysotile as well as the contaminant mineral phases that could not be removed. Therefore, significant compositional differences between the asbestos portion of various chrysotile samples may be obscurred by disseminated, separate mineral phases. There undoubtedly are some significant differences in chemical-physical properties of the chrysotiles derived from different sources that have given rise to the vastly different gross appearances, but these appearances have not been or cannot be readily quantified. While significant differences in the weight losses of chrysotile with time in aqueous suspensions, pH of chrysotile-water suspensions, hydroscopicity, etc. have been reported [14], there is no reporting of how these differences relate to chemical composition of the chrysotile phase vs. quantities and compositions of non-chrysotile impurities. Since these factors, especially the weight loss in water and hydroscopic tendencies, are of great importance in quantitative analyses, particularly where the quantitation is dependent upon preparation of a calibration curve, determinations of chemical compositions as well as these types of physical properties must be made for the various chrysotile asbestos types expected to be encountered in analyses of samples collected in the U.S. Determination of significant differences in these properties between chrysotile specimens will clearly point to the necessity of preparing multiple chrysotile standard reference samples.

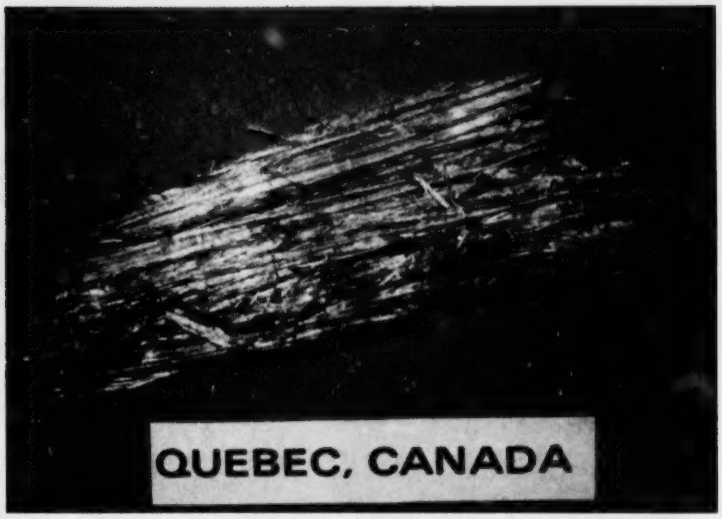
QUEBEC, CANADA

Figure 1. Chrysotile bulk sample (Quebec, Canada).

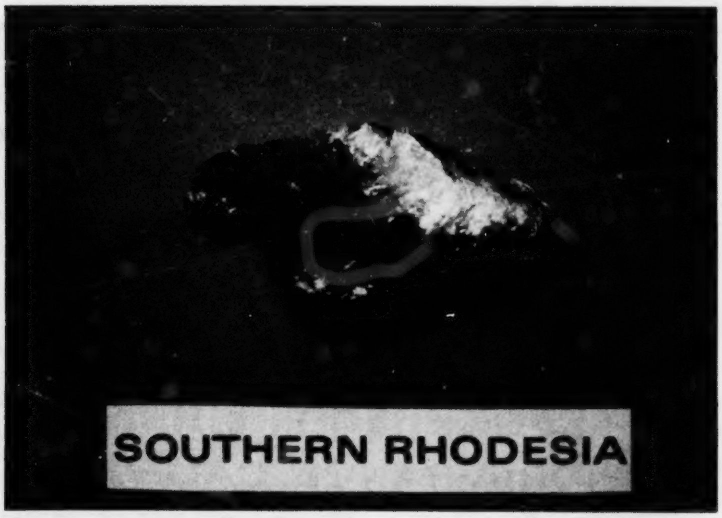
SOUTHERN RHODESIA

Figure 2. Chrysotile bulk sample (Southern Rhodesia).

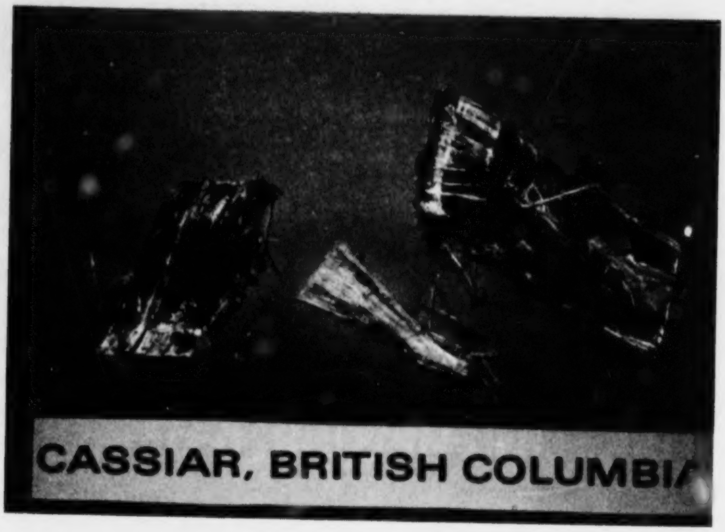
CASSIAR, BRITISH COLUMBI

Figure 3. Chrysotile bulk sample (Cassiar, British Columbia).

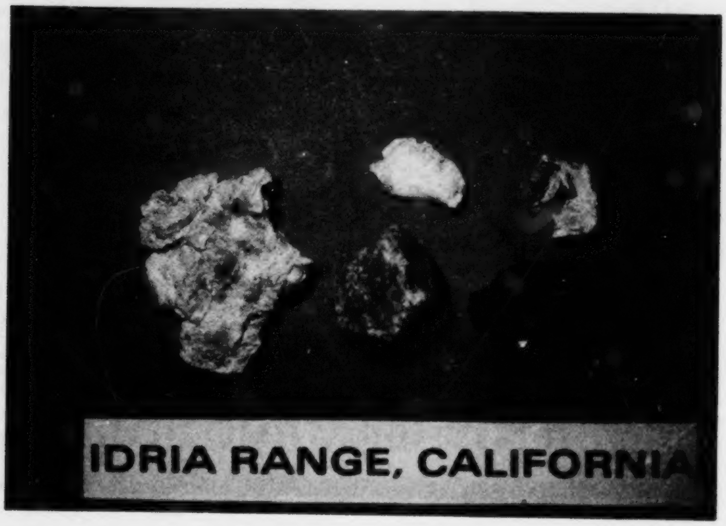
IDRIA RANGE, CALIFORNI

Figure 4. Chrysotile bulk sample (Idria Range, California)

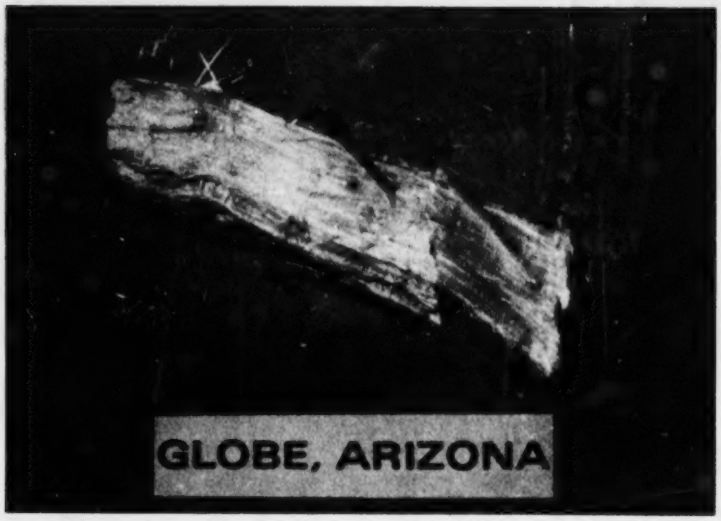

Figure 5. Chrysotile bulk sample (Globe, Arizona).

It is probable that a minimum of two chrysotile standard reference materials (each in several different forms) will have to be prepared to adequately serve the needs of the analytical community. Approximately 85-90 percent of the chrysotile imported into the U.S. is derived from the eastern Canada deposits [13], and therefore chrysotile specimens from the several eastern Canada geographic locales should first be evaluated for selection of one of the chrysotile standard reference materials. A second chrysotile standard reference material should be prepared from the Idria Range (California) massive serpentine fiber deposits because this asbestos deposit is considered unique amongst the commercially developed asbestos deposits worldwide [13].

Consideration should also be given to the preparation of the nonfibrous counterparts of the asbestos mineral types. Methods must be developed for distinguishing amphibole cleavage fragments from true amphibole asbestos. Although the existing federal government definition of "fiber" simply avoids the question of distinction between true amphibole asbestos fibers and amphibole cleavage fragments, it is conceivable that on-going bioassay studies will demonstrate that the cleavage fragments fitting "government fiber" definitions pose no health risks. Therefore, analytical methods for distinguishing between amphibole asbestos fibers and amphibole cleavage fragments may have to be developed. The problem of distinguishing between cleavage fragments and asbestos fibers of amphiboles is especially difficult for microscopic particles as figures 6 through 9 clearly demonstrate.

Processing steps in the preparation of asbestos standard reference samples must be selected with respect to the forms in which the samples must be prepared and with respect to the analysis techniques that require standards. For example, comminuted asbestos standards are required for electron microscopy and x-ray diffraction analyses. However, since crystallinity is a physical property of asbestos which is utilized in both electron microscopy and x-ray diffraction identification of asbestos, care must be taken in the comminution process to not alter or reduce the bulk sample as well as individual fiber crystallinity. Chrysotile is especially susceptible to crystallinity alteration by grinding [15,16].

Table 5. Composition for Some Typical Chrysotiles.

Component	Thetford, King Beaver Mine [14]	British Columbia, Cassiar [14]	Russia, Abest [14]	Rhodesia, Shabani [14]	Swaziland, Havelock Mine [14]	California, New Idria [11]	Quebec, Jeffrey Mine [11]	Arizona, El Dorado Mine [17]
SiO_3	38.75	40.75	39.00	39.70	39.93	39.77	39.90	
FeO[b]	2.03 (0.32) [17]	0.28 (0.28) [17]	1.53	0.70 (0.45) [17]	0.45	ND[a]	ND[a]	0.05
Fe_2O_3[b]	1.59 (5.06) [17]	0.44 (2.75) [17]	0.54	0.27 (3.85) [17]	0.10	2.02	2.93	0.51
Al_2O_3	3.09	3.37	4.66	3.17	3.92	0.66	1.47	
CaO	0.89	0.35	2.03	1.08	1.02	0.32	0.05	
MgO	39.78	41.28	38.22	40.30	40.25	40.62	40.26	
MnO	0.08	0.03	0.11	0.26	0.05	0.07	0.06	
Na_2O	0.10	0.07	0.07	0.04	0.09	0.01	0.04	
K_2O	0.18	0.04	0.07	0.05	0.09	ND[a]	0.08	
H_2O^+	12.22	12.86	11.37	12.17	12.36	12.69	12.81	
H_2O^-	0.60	0.78	0.77	0.64	0.92	1.54	1.17	
CO_2	0.48	0.44	1.83	2.13	1.04	0.78	0.51	
Total	99.79	100.69	100.20	100.51	100.22	98.48	99.28	

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[a] ND = not determined.

[b] Iron values in parentheses were reported in reference 17 to illustrate the differences in iron contents amongst chrysotile from various sources.

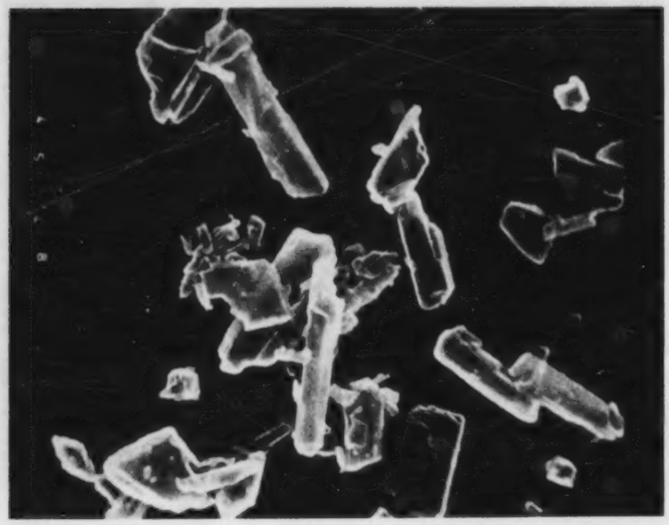

Figure 6. Ground powder form of a grunerite that is classified as a nonfibrous grunerite in the bulk sample form. (Secondary electron image, 3,000X).

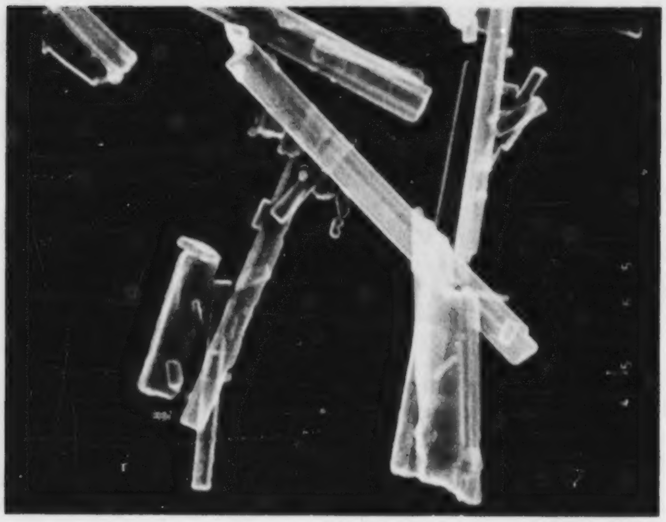

Figure 7. Ground powder form of a grunerite that is classified as a fibrous grunerite (asbestos) in the bulk sample form. Blocky, non-parallel sided particles are observed in this sample as well as the nonfibrous sample. (Secondary electron image, 10,000X).

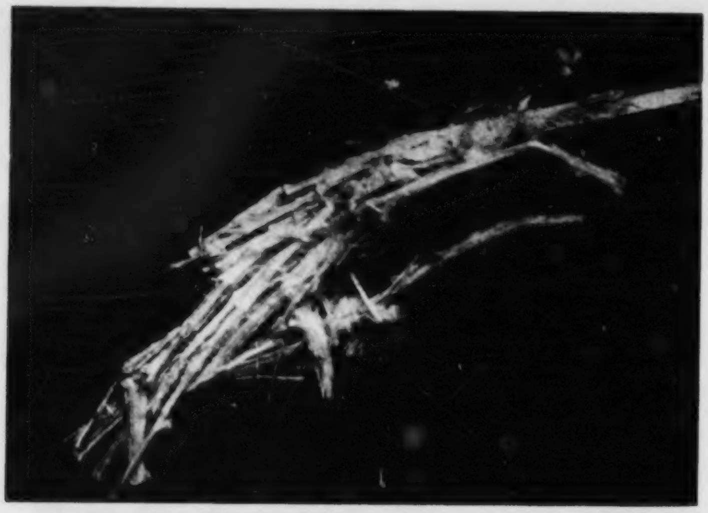

Figure 8. Bulk sample of obviously fibrous tremolite (photomicrograph, 1.8X).

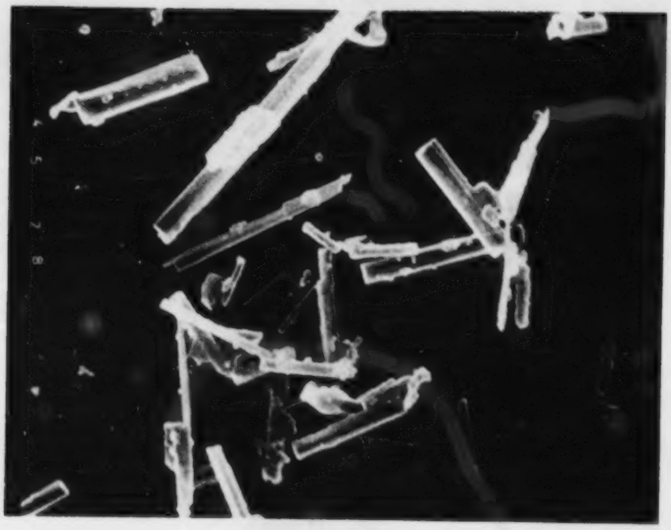

Figure 9. Ground powder of the fibrous tremolite depicted in figure 8. Many fibers do not have parallel sides. (Secondary electron image, 3,000X).

Particle sizes required of asbestos standard reference materials for electron microscopy and x-ray diffraction bulk powder and thin film samples are quite small. In the interest of preserving materials, preventing crystallinity alterations and contamination during grinding procedures, a serial grinding-separation procedure is recommended, once beneficiation and other raw sample clean-up processings have been completed. That is, rather than attempting to grind an entire portion of the sample to the desired size range, grinding should proceed for a short period of time after which the desired size material is removed from the mixture of partially ground sample (e.g., by flotation); the oversize material is then returned to the grinder and ground for a further short period of time. The processes of grinding and removal of the fines, though slow, minimizes heat build-up in the sample that could result in crystallinity alteration and also minimizes tangling of the fibers into non-dispersable balls [7].

The grinding-separation process also provides for simultaneous preparation of the various size ranges of materials required by different analytical techniques. For example, the coarser fibers required by polarized light microscopy analysis techniques could be the remaining "oversized" material after the first one or two grinding-separation steps. By preparing the various size ranges required from one starting material, ambiguities in the sameness of two asbestos samples from different sources is eliminated.

The chrysotile and tremolite bulk samples and ground asbestos samples portrayed in the photographs were collected and processed under NIOSH Contract No. 210-75-0043. Mr. George Yamate of IIT Research Institute provided the scanning electron micrographs of the tremolite and grunerite samples.

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GLASS AS A MATERIAL FOR ASBESTOS STANDARDS

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Abstract

Glasses are finding increased use as analytical standards, particularly for micro-analytical techniques, because they can be produced with a broad composition range and excellent homogeneity and durability. The nature of glass also allows the production in several forms, e.g., bulk, spheres, or fibers.

Glass may prove useful in two areas of asbestos standards, for chemical composition and for physical dimensions.

Some compositions of interest in asbestos studies have been produced as glasses. Others cannot be made as glasses without additions to aid particular properties, such as to reduce melting temperatures or decrease the crystallization tendency. It does appear several compositions of interest could be produced as homogeneous glasses for use as composition standards if desired.

For size standards it would be desirable to have fibers of carefully controlled diameter and length in the range of 0.1 μm diameter and 5 μm long. Suitable fibers for producing such standards are not currently available. However, the theory and experimental work on fiber drawing suggest that it may be possible to alter present techniques to produce such fibers.

1. Introduction

Glass has found increasing use in recent years as a material for a variety of standards, particularly as analytical standards for microanalytical techniques. The use of glass for such standards is related to some of the unique properties of the glassy state.

Glass has been defined in a number of different ways; in structural terms it can be defined as a non-crystalline solid. That is, glass lacks the long range repetitive order of a crystal. Figure 1 shows a schematic view of a glass and a crystal produced from the same structural units. The glass shows a more or less random orientation of structural units while the crystal is constructed of the same structural units in a specific, repeated orientation.

The glassy state is always metastable in terms of thermodynamics. A material can lower its free energy by going from the disordered glassy state to an ordered crystalline arrangement. The kinetics of the transition from liquid to crystalline structure control whether a given material can be obtained in the glassy state. For a wide variety of materials the nucleation and crystallization behavior is such that it is possible to avoid crystallization on cooling from the melt and thus produce glasses that are stable, with respect to crystallization at room temperature, for an indefinite period of time.

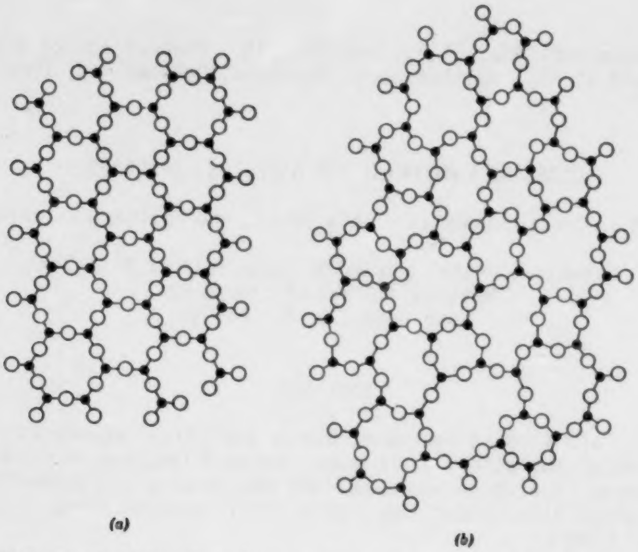

Figure 1. (a) Regular crystalline lattice, and (b) corresponding irregular glass network.

Figure 2 shows a plot of specific volume vs. temperature for a glass and a corresponding crystalline material. When crystallization occurs on cooling a melt to its liquidus or crystallization temperature, there is an abrupt volume change at the transition from the liquid to crystalline state due to the related structural differences. In the case of glass formation there is no sudden structural change, the volume decreases smoothly and continuously even below the liquidus temperature. At a point near where the melt becomes essentially a solid (viscosity of 10^{13} poise) there is a change in slope in the V vs. T curve. This point is called the glass transition point, Tg. Below Tg the material is considered a solid, above Tg the material is considered a liquid. The material would be considered a glass only below the glass transition temperature.

Materials which can be formed as glasses generally show a relatively high viscosity at their liquidus temperature. This is a major factor that results in nucleation and crystallization behavior such that the melt can be brought through the crystallization region without crystals being formed. Materials which cannot be formed as glasses or only with great difficulty generally show a low viscosity at the liquidus temperature. The viscosity of a material during glass formation increases smoothly as temperature is decreased, this allows the use of a number of different forming techniques for producing glasses in a variety of shapes directly from the melt.

This brief overview of the glassy state points out some of the advantages of glasses as materials for standards and also some of the problems that must be considered. Synthesizing a glass, one starts with a melt, that is with a liquid which can be homogenized by stirring. This high homogeneity is retained when the melt is cooled into the glassy state. The random structure of a glass allows a wide variety of ions to be incorporated into a glassy matrix and still maintain the homogeneity required. In a crystalline material the type of sites available and ions that can be accommodated are usually quite limited. In a glass, because of the disordered structure no specific sites exist but rather the structure can change and accommodate a variety of ions. This is particularly useful when producing standards for analytical use where a number of elements are desired in a single matrix. The problems of partitioning between different crystalline phases or grain boundry segregation that occur in multicrystalline materials are avoided by using a glass.

As mentioned above, glasses are not thermodynamically stable and not all materials or compositions will form glasses. This presents a problem when attempting to produce a glass to simulate the composition of certain crystalline materials.

The smooth increase in viscosity as temperature decreases allows the liquid forming of glass by casting, pressing, drawing, or blowing. Therefore, it is possible to produce

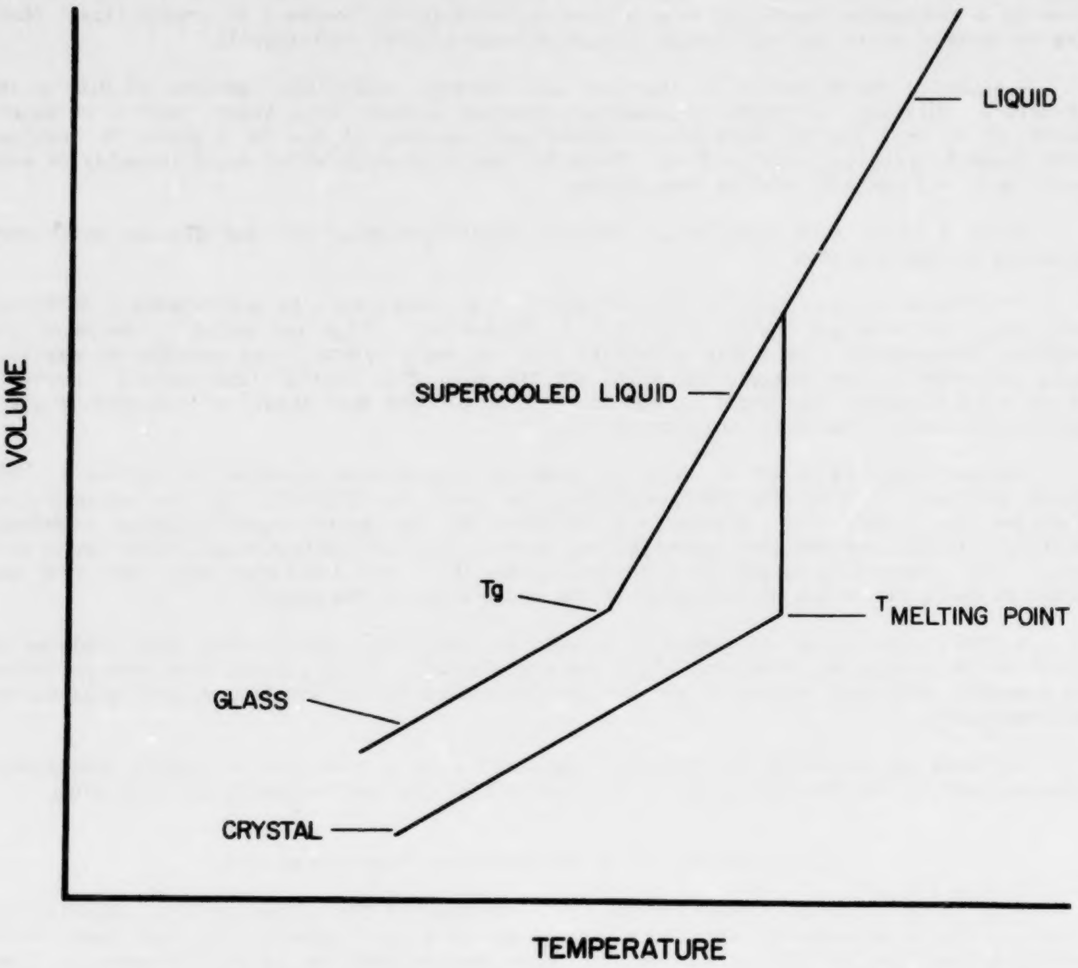

Figure 2. Plot of specific volume vs. temperature for glass and crystalline material.

standards in a variety of forms, e.g., bulk form, spheres, fibers, or thin films. This can be particularly useful for producing analytical standards for certain microanalytical techniques.

2. Glass for Use as Asbestos Standards

There are two different areas where glass may prove useful as a material for standards related to asbestos characterization. The first as standards for chemical composition, the second as standards for fiber dimensions.

2.1 Chemical Composition Standards

There are numerous minerals that are of interest in asbestos studies covering a wide compositional range. Since it is desired to simulate a typical mineral the major as well as minor constituents are specified. This brings up the problem, as mentioned above, that not all compositions will form a glass. However, the asbestos compositions contain significant amounts of SiO_2, which is an excellent glass former and it is frequently possible to produce glass near the composition of interest by making minor composition alterations. Such alterations frequently include additions to reduce the melting temperature to a reasonable level and alterations to decrease the tendency to crystallize. There are few general rules and each composition must be considered individually.

Frequently the minerals of interest will contain significant amounts of H_2O in the structure. Glasses, as normally produced, however contain only trace amounts of water. While it is possible to incorporate significant amounts of H_2O in a glass it involves considerable technical difficulties. Therefore one alteration which would normally be made would be to exclude H_2O from the composition.

Table 1 gives some examples of typical asbestiform materials and glasses which were produced to simulate them.

To produce a glass to simulate chrysotile the composition is approximately 50-50 MgO and SiO_2, on a weight basis since H_2O is eliminated. Li_2O was added to decrease the melting temperature. This was effective but the melt crystallized rapidly on casting. B_2O_3 was added to the second trial melt, but the melt still crystallized rapidly. However, using a rapid quench technique it was possible to produce thin sheets of homogeneous glass having the composition shown in column 3.

Another composition of interest is that of crocidolite as shown in column 4. The glass produced to simulate the composition is shown in column 5. It was necessary to decrease the total iron content and increase MgO to avoid crystallization problems. Addition of TiO_2 and MnO were requested and do not materially affect glass formation in this case. The composition given is a nominal composition and indicates only that iron was added as Fe_2O_3 and is not an indication of the redox state of the glass.

A third composition of interest is that of tremolite. Two glasses were produced to simulate this material, one with Al_2O_3 and one without. These glasses have been certified as Standard Reference Materials and are available from NBS as SRM 470, mineral glasses for microanalysis.

In terms of standards for chemical composition it is possible to produce homogeneous glasses closely simulating many abestiform compositions for use as analytical standards.

2.2 Glass for Use as Standards for Fiber Dimensions

This is an area where glass may be useful because of the unique forming properties of glass. This is an area of importance because of the work of Stanton [1][1] and others which suggests that the ability of a fiber to cause mesothelioma may be related more to fiber geometry than fiber chemistry, at least for inorganic oxides. From this standpoint it would be desirable to have fibers of carefully controlled diameter and length for use as standards in materials characterization work. If such fibers can be produced they would also be valuable for use in medical testing as a further test of Stanton's suggestion since a variety of fibers having a carefully controlled diameter and length, but identical chemistry, have not previously been available. It would be desirable to have fibers approximately 0.1 μm in diameter and 5 μm in length.

To produce the desired fibers it is necessary to have a method not only to produce fibers with close control of diameter but also a method to accurately cut and size the fibers to length. A method has been developed, by the Thermal Insulation Manufacturers Association [2], to cut continuous glass fibers into the length range desired. The method has been used successfully to prepare fibers for animal studies, using fibers of 1-2 μm diameter. Therefore, if fibers of 0.1 μm diameter were available there is reason to believe they could accurately be sized with respect to length.

Unfortunately continuous glass fibers are not currently available below 1 μm diameter. Discontinuous glass fibers can be produced having diameters down to 0.1 μm diameter. These fibers are not suitable for use as size standards since the diameter varies along the

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[1] Figures in brackets refer to the references at the end of this paper.

Table 1. Composition of Typical Asbestiform Materials and Glasses Produced to Simulate Them.

	1	2	3	4	5	6	7	8
	Chrysotile			Crocidolite		Tremolite		
	Typical Analysis	Glass Simulation	Glass Simulation	Typical Analysis	Glass Simulation	Typical Analysis	Glass Simulation	Glass Simulation
SiO_2	41.80	42.00	40.00	51.94	54.00	56.24	54.30 ± 0.20	45.35 ± 0.20
TiO_2	0.05				0.70	Tr.		
Al_2O_3	0.003			0.20		2.28		9.27 ± 0.20
FeO	0.05			19.39			14.42 ± 0.20	9.96 ± 0.20
Fe_2O_3				18.64	25.00	0.07		
Cr_2O_3						0.08		
MnO	0.04				0.50	Tr.		
MgO	42.84	42.00	40.00	1.37	10.00	22.89	14.67 ± 0.20	19.93 ± 0.20
CaO	0.10			0.19	0.50	12.01	15.47 ± 0.20	15.25 ± 0.20
Na_2O	0.03			6.07	8.80	0.86		
K_2O	0.01			0.04	0.50	0.38		
H_2O^+	14.04			2.58		1.59		
H_2O^-	0.28			0.31		0.24		
SO_3	0.09							
CO_2	0.01							
Li_2O		16.00	10.00					
B_2O_3			10.00					

length and the fibers are randomly oriented as a result of the manufacturing process. These drawbacks make it extremely difficult to produce the sharp size distribution required for standards or medical studies.

However, an examination of the theory and practice of producing continuous glass fibers suggests that alterations to current methods may allow the production of continuous fibers in the desired diameter range. A theory of the drawing process developed by Bruckner and Stehle [3] predicts that using a simple bushing system, as is normally used, the minimum fiber diameter attainable is approximately 1.5 µm. This is in excellent agreement with experimental results of fiber drawing. A simplified drawing configuration is shown in figure 3. It consists of the bushing or orifice plate, through which the glass flows, and a winding mechanism. A glass fiber is pulled from the orifice and attached to the drum which is then rotated thus drawing the fiber. The flow rate of glass through the orifice is independent of drawing speed of the drum. Therefore independent control of the glass flow rate and the winding speed determine the final fiber diameter, if breakage does not intervene. Such a drawing process provides very close control over the fiber diameter.

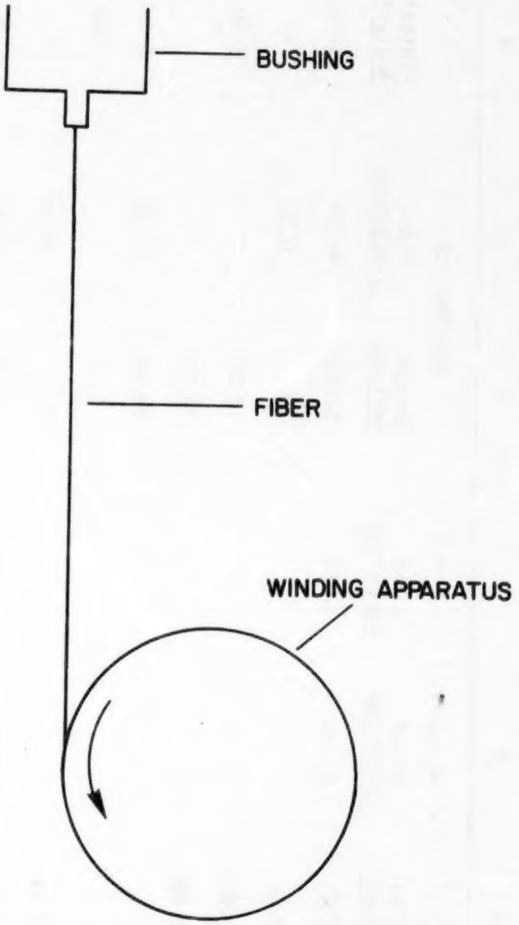

Figure 3. Schematic representation of apparatus for drawing continuous fibers.

Studies have been carried out to understand the hydrodynamics of the drawing process and to determine critical engineering parameters. These studies point out several areas of present practices that might be altered in an effort to produce submicron fibers. To model the drawing process the glass stream is divided into three separate areas as shown in figure 4. The upper jet region is important not for attenuation, only a factor of 5-10, but as a source of instability in the process. The instability is caused by surface tension which tries to pinch off the glass stream. The surface tension forces have to

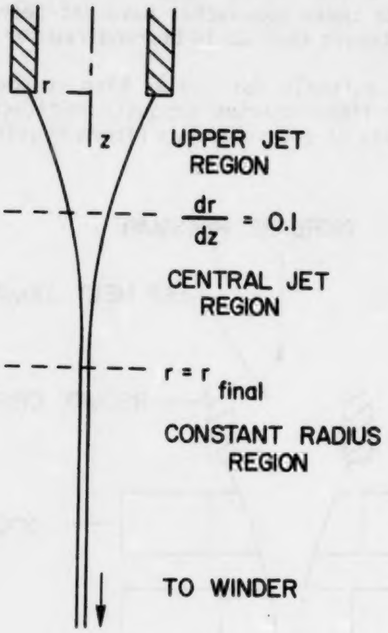

Figure 4. Glass Stream

overcome the viscosity of the glass and it is therefore desirable to have the viscosity of the glass as high as possible at this point. However, the viscosity must be sufficiently low to sustain flow through a small orifice. Therefore it is desirable to have the viscosity increase rapidly with the distance from the orifice.

The central jet region is where most of the attenuation occurs, normally a factor of 50 to 100. To produce very fine fibers it is desirable to have the incoming diameters as small as possible to decrease the necessary attenuation. It is further desirable to have the viscosity low at the start of the region and change slowly with temperature to produce a long zone of gradual attenuation.

In the constant radius region there is no further attenuation but breakage can occur before the fiber reaches the drum. Air turbulence caused by fiber/air and drum/air friction as well as fiber vibration contribute to breakage of the solidified fiber.

It can be seen from this brief description that different conditions are required in each section. It is not possible to optimize the conditions required to produce submicron fibers solely through the use of a simple bushing, natural cooling, and glass selection. A more promising approach is to attempt to produce the desired conditions in each section by controlling the temperature distribution of the glass stream. To increase the stability in the upper jet region the viscosity should be as high as possible consistent with maintaining glass flow. This can be achieved by low melt temperatures within the crucible and by cooling the glass stream immediately as it leaves the orifice. To provide sufficient glass flow with low melt temperatures it may be necessary to increase hydrostatic melt pressure at the orifice. This can be done by either a high hydrostatic column or by applying gas pressure at the melt surface.

The attenuation zone could be lengthened through the use of auxiliary heaters.

Observations of the drawing process also indicate that the finer the initial diameter the smaller the final fiber diameter. Very fine orifices may prove useful, though again pressurization may be required to control glass flow. These alterations are shown schematically in figure 5.

Experimental evidence exists which suggests that each of these modifications can be effective. However, to date these approaches have not been combined in a system to produce the submicrometer diameter fibers that would be required for asbestos size standards.

In conclusion, fibers suitable for use as size standards are not currently available. The theory and practice of fiber drawing suggests modifications to current practices that could result in the production of the submicron fibers required.

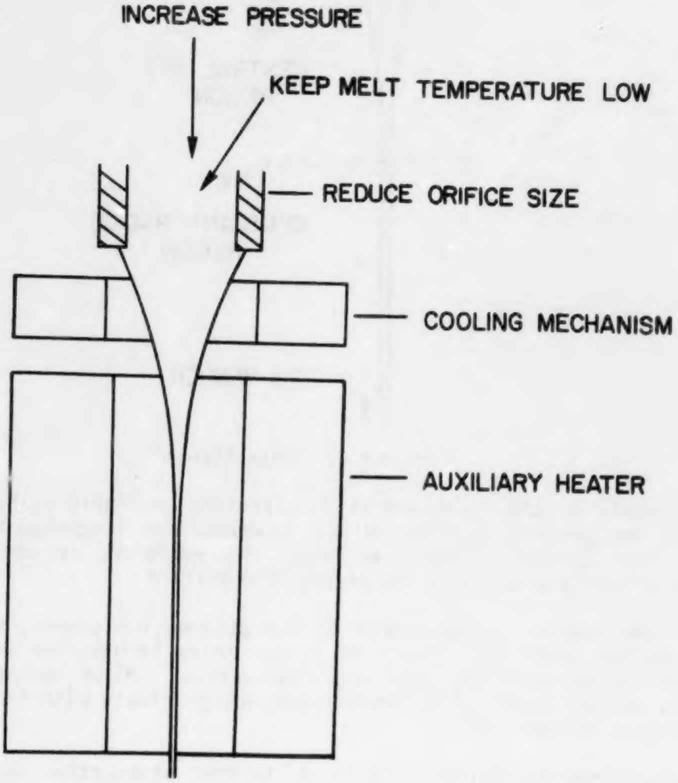

Figure 5. Schematic representation of suggested alterations to the drawing apparatus for the production of submicrometer fibers.

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THE NBS PROGRAM FOR STANDARD REFERENCE MATERIALS

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The Standard Reference Materials (SRM's) program will be described and specific examples given of SRM's and how they aid in measurement compatibility and traceability in the environmental area. An SRM is a material or device for which a chemical or physical property has been certified by the National Bureau of Standards (NBS). SRM's can be used to calibrate measurement systems, to evaluate measurement methods, and to provide traceability of the measurement to NBS. In general, measurements are made at NBS or in cooperating laboratories in such a way that accurate values for the property are obtained and the present best estimate of the true value and its uncertainty are certified. Current SRM's in the environmental and industrial hygiene areas include analyzed gases for atmospheric pollutants such as SO_2 and NO_2; lead, sulfur and mercury content in fuels and water; quartz, beryllium, and other metals on filters to be used in determining their level in an industrial atmosphere; and trace element concentrations in coal, fly ash, fuel oil, urban aerosols, and water.

The National Bureau of Standards (NBS) has issued Standard Reference Materials (SRM's) since 1906. During most of these years the emphasis has been on metallic compositional reference materials. During the last 10 years, however, there has been a large increase in requests for SRM's in clinical chemistry, nuclear materials, physical science, and electronics as well as in environmental and occupational health areas. Some examples of SRM's in the environmental and industrial hygiene areas are shown in Table 1. Although the concentrations of more than one element are generally certified in these SRM's the concentration of lead, which is of particular interest to the health of individuals, has been indicated in this table.

Table 1. Some Examples of Standard Reference Materials for Use in The Environmental and Industrial Hygiene Areas.

SRM	Material	Certified	Lead (μg/g)
1643a	Water	17 trace elements	0.0027
1577	Bovine Liver	16 major and trace elements	0.34
1566	Oyster Tissue	19 minor and trace elements	0.48
1635	Subbituminous Coal	14 trace elements	1.9
1632a	Bituminous Coal	18 trace elements	12.4
1636a	Lead in Reference Fuel	lead only, 4 levels	11.2 to 76.4
1633a	Coal Fly Ash	20 major and trace elements	72.4
1645	River Sediment	13 major and trace elements	714.0
1648	Urban Particulates	9 elements	6550.0
2676a	Metals on Filter Media	4 elements, 3 levels	6.9 to 29.64 μg/filter
2661b	Benzene on Charcoal	16 to 54 μg/tube, 4 levels	
1661-1664	SO^2 in N^2 for Stack Gas	480 to 2521 ppm	

Standard Reference Materials are well-characterized, homogeneous, stable materials or simple artifacts with specific properties that have been measured and certified by NBS. They are used to help develop test methods and to calibrate measurement systems. By using SRM's the long-term reliability and integrity of measurement processes and the development and enforcement of equitable regulations can be assured.

Two general procedures are followed in the certification of an SRM:

1. Measurement by a so called "definitive" method of known accuracy having very small systematic errors.

2. Measurement by two or more independent methods having systematic errors that are estimated to be small relative to the desired certification accuracy. Measurements are generally made at NBS but may be made by a group of cooperating laboratories, preferably using previously issued SRM's as controls.

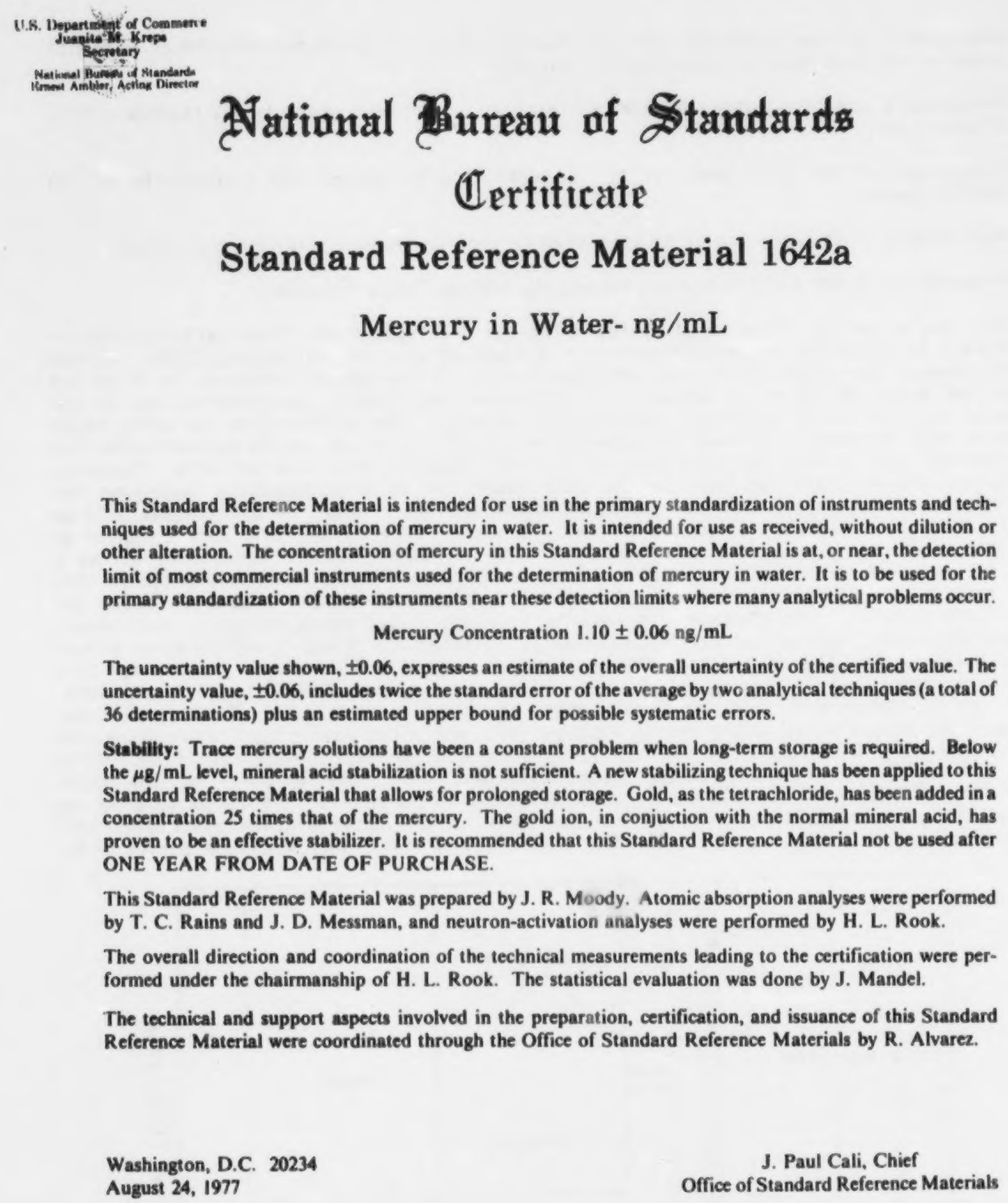
When a user purchases an SRM, a certificate is provided (see figure 1) with the material that clearly states the certified value(s), the uncertainty of the value(s), directions for using the material, limitations on its use, and information on its possible instability. The certified value is the present best estimate of the "true" value and is not expected to deviate from the "true" value by more than the stated uncertainty. Ideally the uncertainty includes the systematic error of the measurement process, the measurement imprecision, and the material inhomogeneity.

The difference between primary reference materials (SRM's) and secondary or working reference materials should be made clear. While both should be homogeneous and stable, the working standards should relate very closely to the real world and should as much as possible match the material being measured. Working standards are not only more economical but if they are carefully calibrated they can very often lead to a more accurate calibration of the measurement system. The SRM on the other hand should be free of unnecessary complications and unambiguity in the certified property. A good example of this difference is found in the determination of the level of an anticonvulsent drug in a patient's blood. The method generally used in a clinical laboratory can be very inaccurate so that calibration is needed. Working standards can easily be made in the clinical laboratory by spiking whole serum with the drug at several weighed-in levels. The drug and the whole serum, however, can have interferences that still make the calibration questionable. To help this measurement problem, SRM 900 was prepared with four high-purity drugs and a specially processed serum so that all interferences were removed. After preparation the concentrations of the drugs were determined by using two independent methods which gave excellent agreement. This SRM can now be used sparingly by a clinical laboratory to calibrate its working standards and to verify the measurement procedure.

The role of the Office of Standard Reference Materials is to provide funds and directions for the development, procurement, and certification (measurement) of SRM's. Funds for these activities come from regular appropriations and the NBS Working Capital Fund. Monies used for the direct production of an SRM are returned to the Working Capital Fund as the units are sold. A surcharge on each unit of an SRM provides funds for the operation of the office which includes packaging, storage, inventory, sales, and shipping as well as the management of the program.

Criteria for the production of an SRM include a positive evaluation of its technical impact on a measurement system, the user's acceptance and support, material availability in suitable form and as a homogeneous lot, and the lack of any serious technical risk in its preparation and certification. The production cycle for an SRM typically takes about two years for planning and development and two years for preparation and certification. This cycle includes the following steps:

1. Identification of the needs of a measurement system. (In the case of asbestos detection and analysis there seems to be a need for a bulk powder for assurance checking of overall sample handling and measurement techniques for both chrysotile and amphibole forms, for powder in solution to evaluate sample preparation, and for fibers on a filter to qualify a TEM and the operators.)



U.S. Department of Commerce
Juanita M. Kreps
Secretary

National Bureau of Standards
Ernest Ambler, Acting Director

National Bureau of Standards

Certificate

Standard Reference Material 1642a

Mercury in Water- ng/mL

This Standard Reference Material is intended for use in the primary standardization of instruments and techniques used for the determination of mercury in water. It is intended for use as received, without dilution or other alteration. The concentration of mercury in this Standard Reference Material is at, or near, the detection limit of most commercial instruments used for the determination of mercury in water. It is to be used for the primary standardization of these instruments near these detection limits where many analytical problems occur.

Mercury Concentration 1.10 ± 0.06 ng/mL

The uncertainty value shown, ±0.06, expresses an estimate of the overall uncertainty of the certified value. The uncertainty value, ±0.06, includes twice the standard error of the average by two analytical techniques (a total of 36 determinations) plus an estimated upper bound for possible systematic errors.

Stability: Trace mercury solutions have been a constant problem when long-term storage is required. Below the μg/mL level, mineral acid stabilization is not sufficient. A new stabilizing technique has been applied to this Standard Reference Material that allows for prolonged storage. Gold, as the tetrachloride, has been added in a concentration 25 times that of the mercury. The gold ion, in conjuction with the normal mineral acid, has proven to be an effective stabilizer. It is recommended that this Standard Reference Material not be used after ONE YEAR FROM DATE OF PURCHASE.

This Standard Reference Material was prepared by J. R. Moody. Atomic absorption analyses were performed by T. C. Rains and J. D. Messman, and neutron-activation analyses were performed by H. L. Rook.

The overall direction and coordination of the technical measurements leading to the certification were performed under the chairmanship of H. L. Rook. The statistical evaluation was done by J. Mandel.

The technical and support aspects involved in the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by R. Alvarez.

Washington, D.C. 20234
August 24, 1977

J. Paul Cali, Chief
Office of Standard Reference Materials

(over)

Figure 1. Example of an SRM certificate.

2. Development of the candidate SRM and resolution of all problems associated with its preparation, homogeneity, measurement, and use.
3. Procurement and preparation of the material in sufficient quantity to provide a 5 to 10 year supply.
4. Evaluation of the bulk material or the artifacts to assure the homogeneity of the entire supply.
5. Measurement of the property to be certified to provide the basis for certification.
6. Preparation of the certificate and making the SRM available for sale.

This cycle can be illustrated by following the development and final certification of an artifact to calibrate the magnification of a scanning electron microscope (SEM). It had been documented (by about 1970) that the uncertainty in the magnification scale of an SEM was on the order of 10 to 30 percent. To calibrate this scale, laboratories had to use artifacts such as ruled gratings and wire mesh screens. The difficulties in using these artifacts were in preparing them for viewing in an SEM, in accepting an average value for the spacing, and in the variable and ill-defined image of the line or wire. Research started at NBS in 1973 resulted in the development of an electroplating technique for building up various thicknesses of nickel between very thin layers of gold (about 0.06 µm thick). When this sandwich structure is cut and viewed edge-on the gold layers appear as very thin straight lines. Problems that had to be overcome included uniformity across a sheet, delamination of the layers, gas bubbles, and strain. Production was started in 1976 which resulted in the successful preparation of a composite sheet from which about 200 pieces were sheared. Each piece was mounted in a copper filled epoxy inside a short length of stainless steel tubing and metallographically polished. A Knoop identification placed on the specimen is used to identify the measurement area (See figure 2). Patents for this device and process were issued to Ballard, Ogburn, and Young of NBS in 1978 and 1979. Evaluation of homogeneity and measurement for certification were made at the same time. Each unit was measured by comparing to a master unit which had been directly measured with an interferometry technique. Nominal 1, 2, 3, 5, and 50 µm spacings are certified. Units which had unacceptable line spacings were discarded. For this initial lot the total uncertainty for each spacing of 1 through 5 µm was 0.04 µm and for the 50 µm spacing was 0.48 µm. These uncertainties were the linear sums of the uncertainties associated with the calibration of the master unit and the comparison of each unit with the master unit.

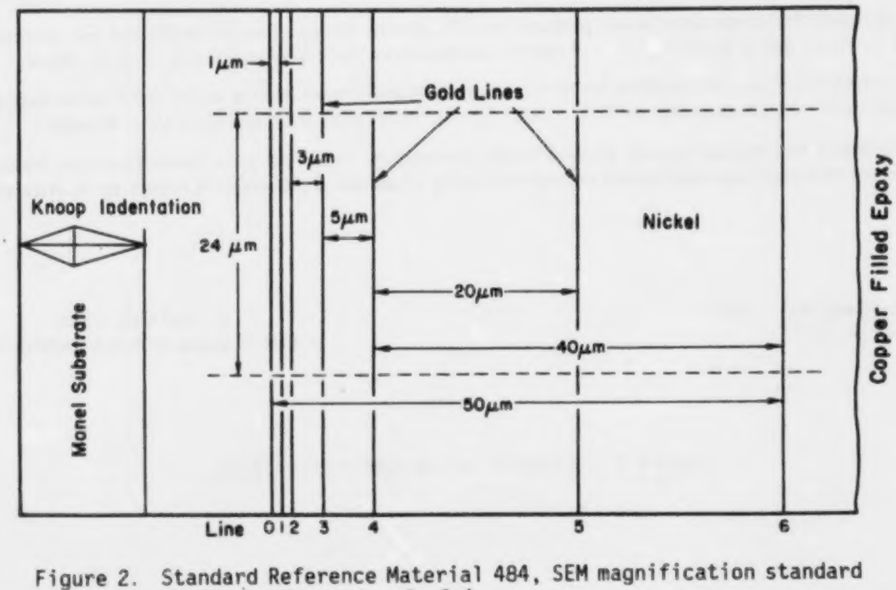


Figure 2. Standard Reference Material 484, SEM magnification standard (A Stage Micrometer Scale).

This Standard Reference Material, designated as SRM 484, can be used to calibrate the magnification scale of an SEM to an accuracy of five percent or better in the range of 1000 to 2,000X. One hundred and fifty units of this SRM went on sale in 1977 and were sold out within six months. Since then, SRM 484a (the second lot) has been certified and nearly sold out while the third lot is in the process of being certified.

Not all SRM's that are useful in the verification of measurement systems have a chemical or physical property that is certified. Examples are SRM 1010, the Microcopy Resolution Test Chart and SRM 469, the SEM Resolution Test Sample. SRM 1010 contains 26 5-line patterns (see figure 3) and is only certified to have high contrast and to be defect free.

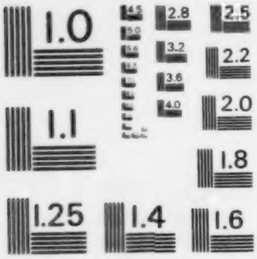


Figure 3. Standard Reference Material 1010 (Microscopy Resolution Test Charts). This Standard Reference Material is intended to be used to determine the resolving power of microscopy systems. It meets all of the requirements for ISO Test Chart No. 2, as described in International Standard ISO 3334-1976(E).

In summary it can be noted that a compatible measurement system contains at best five components:

1. A uniform system of units;
2. Primary and secondary reference materials;
3. Definitive and reference methods of measurement;
4. Field methods of measurement;
5. Long term stability and quality assurance.

Ideally, a definitive measurement method is used to certify an SRM which in turn is used to develop and calibrate reference methods and secondary standards. These in turn are used to calibrate field methods of measurement. The long term availability of the SRM from NBS assures the continued quality control of the system.

National Bureau of Standards Special Publication 619. Proceedings of the NBS/EPA Asbestos Standards Workshop held at NBS, Gaithersburg, Maryland, October 1-3, 1980. (Issued March 1982)

IDENTIFICATION AND QUANTIFICATION OF ASBESTOS IN CONSTRUCTION MATERIALS USING POLARIZED LIGHT MICROSCOPY: THE NEED FOR STANDARDS

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Abstract

The Bureau of Mines Particulate Mineralogy Unit, in cooperation with the Environmental Protection Agency (EPA), conducted a round robin program to evaluate the reliability of analyses of asbestos-containing building materials by polarized light microscopy. This microscopic technique was selected by EPA as the principal analytical method for asbestos identification and quantification in their program to evaluate the potential health risk from exposure to airborne asbestos in public buildings.

Results of the round robin show a need for monomineralic reference samples of asbestos and nonasbestos components of these materials to aid in identification training. Also necessary are bulk standards containing known amounts of asbestos to be used in verification and quality control of quantification techniques.

1. Introduction

The identification and quantification of asbestos in insulation materials were investigated by the Bureau of Mines Particulate Mineralogy Unit (PMU). One component of the PMU's mission is to provide assistance to other Federal agencies in mineral particulate-related problems. The Environmental Protection Agency (EPA) requested PMU assistance in the evaluation of polarizing light microscopy (PLM) for determination of asbestos in insulation and construction materials in public buildings, particularly schools.

In the guidelines developed by EPA for evaluating the potential health risk from exposure to airborne asbestos in public buildings [1][1], PLM was selected as the principal analytical method for measurement of asbestos in various materials used in construction prior to the EPA ban on the use of sprayed asbestos-containing materials. The choice of PLM was made on the basis of the need to determine asbestos in moderately complex samples, the speed and relatively low cost of the visual method, the wide availability of PLM capability, the sensitivity of the method at very low concentrations, and the ability to distinguish between fibrous and nonfibrous forms of a mineral, for example, chrysotile and antigorite. No other instrumental method such as x-ray diffraction or infrared spectrophotometry offers all of these features.

Since visual observation is the basis of the PLM method, reliability is strongly operator dependent. To obtain a measure of the reliability of the identification and quantification versus operator training and experience, the Bureau of Mines, with assistance from EPA, conducted a round robin PLM evaluation using samples of insulation and related materials collected from various public buildings. The need for suitable standards for PLM analyses is based on the results of this round robin program together with the experience gained in conducting PLM training in asbestos determination for State and Federal personnel. This report consists of a brief summary of the round robin program, followed by discussion of two approaches to the development of PLM standards.

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[1] Figures in brackets refer to the literature references at the end of this paper.

2. Round Robin Study

2.1 Samples

Fourteen samples of insulation materials submitted to the PMU for evaluation were used in the round robin program. The samples were selected to cover a wide range both in asbestos concentration and type of asbestos and in variations in the fillers and binders used in the construction materials. These samples were characterized in detail by Avondale personnel using microscopic and x-ray diffraction methods of analysis. Of the 14 samples, there were four with chrysotile, three with amosite, two with amosite and chrysotile, and one with chrysotile and crocidolite; there was no detectable asbestos in four samples. The asbestos concentration ranged from not detectable to approximately 80 weight-percent. Fibrous nonasbestos constituents included rockwool, fiberglass, and organic fibers.

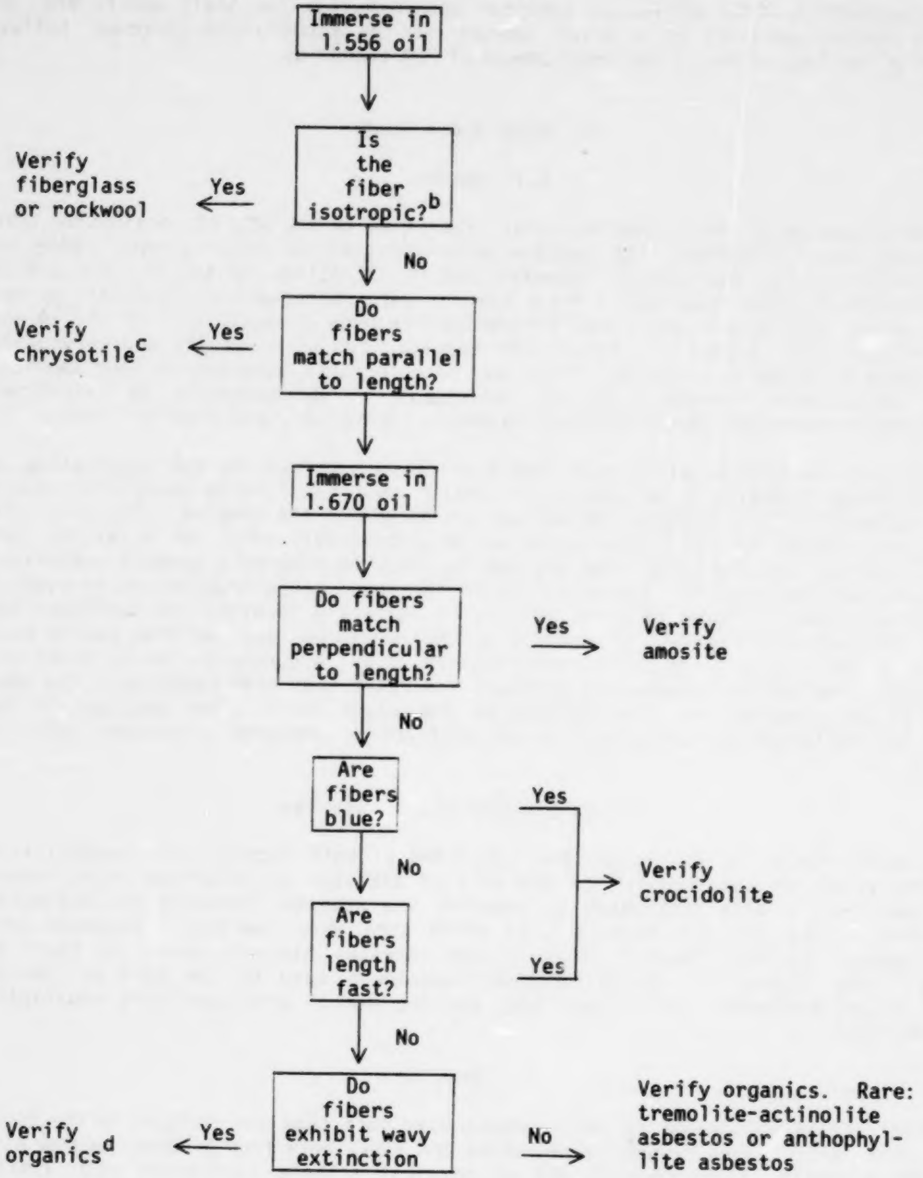
No attempt was made to blend each sample prior to submittal to the cooperating laboratories. Although blending is an essential step in most round robin analytical studies, it was not included in this program because of the nature of the samples. The only effective way to blend samples of this type would be to extensively mill the materials prior to blending; however, this milling step may not be incorporated in a general analytical procedure to be adopted by EPA. The objective of the round robin program was to evaluate the various laboratories in terms of their ability to correctly identify and semiquantitatively determine the asbestos minerals in samples as they would be received from public buildings for analysis rather than to generate concentration values accurate to two or three significant figures. Preliminary studies at Avondale indicated that grab sampling of the materials as received was adequate for the purpose of the round robin. The adequacy of the grab sampling was confirmed by subsequent x-ray diffraction analyses discussed later in this report.

2.2 Participating Laboratories

The participants in the roundrobin consisted of both Federal and commercial laboratories interested or involved in the analysis of asbestos by polarized light microscopy. The 26 laboratories were instructed to identify the asbestos minerals and estimate their concentration using the PLM methods with which they were familiar. Avondale personnel provided guidelines for identification of the asbestos minerals based on their optical properties (see figure 1). Quantification techniques used by the various laboratories included visual estimates, point counting, and the use of grid graticule (multiple point count technique).

2.3 Results

Several of the participating laboratories used more than one analyst in the roundrobin program. The participants ranged in education and experience from graduate degree mineralogists with extensive experience in PLM to physical science technicans with limited PLM training (see Table 1). As expected from this diverse group of participants, there were some obvious difficulties in the identification of the specific variety of amphibole asbestos. For example, low concentrations of crocidolite were incorrectly identified as either amosite, anthophyllite-asbestos, or tremolite-asbestos. In general, from the results of the 33 analysts there were one to two incorrect identifications per sample, and up to four incorrect identifications on one sample. In terms of the EPA proposed algorithm [2] for assessing the potential health hazard, this misidentification is not a serious problem since all of the asbestos minerals are assigned equal rank in the hazard evaluation. This problem with misidentification should be resolved by additional training and experience using reference slides prepared from asbestos and other minerals commonly found in construction materials.


Immerse in 1.556 oil
Is the fiber isotropic?b
Yes
Verify fiberglass or rockwool
No
Do fibers match parallel to length?
Yes
Verify chrysotilec
Immerse in 1.670 oil
Do fibers match perpendicular to length?
Yes
Verify amosite
No
Are fibers blue?
Yes
No
Are fibers length fast?
Yes
Verify crocidolite
No
Do fibers exhibit wavy extinction
Yes
Verify organicsd
No
Verify organics. Rare: tremolite-actinolite asbestos or anthophyllite asbestos

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[a] Verifications should be based on all optical properties.

[b] Fiberglass may be strained. This results in weak birefringence. Fiberglass identification must be checked.

[c] Organics may have the same refractive index as chrysotile.

[d] Chrysotile may appear to possess wavy extinction but should have been identified earlier.

Figure 1. Guideline for petrographic analysis of fibrous constituents commonly found in construction materials[a].

Table 1. Listing of Training, Experience, and Technique of Participants.

Analyst	Training and Experience	Technique
1A	Mineralogist, moderate experience	Polarizing microscope
1B	Mineralogist, moderate experience	Polarizing microscope
1C	Mineralogist, moderate experience	Polarizing microscope
2A	Mineralogist, extensive experience	Polarizing microscope
2B	Mineralogist, extensive experience	Polarizing microscope
3	Mineralogist, extensive experience	Polarizing microscope
4	Metallurgist, limited mineralogy training	Polarizing microscope
5A	Mineralogist, extensive experience	Polarizing microscope
5B	Geologist, limited microscopic experience	Polarizing microscope
6	Mineralogist, extensive experience	Polarizing microscope
7	Physical science technician, extensive mineralogical experience	Polarizing microscope
8	Geologist-biologist	Polarizing microscope
9	Not stated	Dispersion staining
10	Materials scientist	Polarizing microscope
11	Mineralogist, extensive experience	Polarizing microscope
12	Microscopist (novice mineralogist), experienced	Polarizing microscope
13	Chemist, training in microscopy	Polarizing microscope
14	Not stated	Polarizing microscope-dispersion staining
15	Technician, moderate experience	Dispersion staining
16	Mineralogist, experienced	Polarizing microscope
17	Chemical engineer, training in microscopy	Polarizing microscope
18	Mineralogist, experienced; geologist, moderate experience	Polarizing microscope
19	Chemist, training and experience in microscopy	Dispersion staining
20A	Mineralogist, extensive experience	Polarizing microscope
20B	Mineralogist, very limited experience	Polarizing microscope
20C	Mineralogist, very limited experience	Polarizing microscope
21A	Microscopist, extensive experience	Polarizing microscope-dispersion staining
21B	Microscopist, extensive experience	Polarizing microscope-dispersion staining
22	Chemist, limited experience in microscopy	Polarizing microscope-dispersion staining
23	Experience in polarized light microscopy	Polarizing microscope
24	Not stated	Not stated
25	Not stated	Not stated
26	Geologist, moderate experience	Polarizing microscope

The most serious problem noted was the quantification of the asbestos minerals. Table 2 lists the number of participants reporting asbestos concentrations of <1 percent, 1 percent to 10 percent, and >10 percent for each sample. Where a participant listed "trace" concentrations, the estimate was placed in the < 1.0 percent category. When estimates were given as ranges which overlapped two categories, they were placed in the higher concentration category; for example, 5 to 15 percent would be placed in the >10 percent category. If two asbestos types were identified, the percents were summed, and if the ranges were given, the two upper ends of the ranges were summed to get the maximum asbestos estimate.

Table 2. Quantification of Asbestos Content by Polarized Light Microscopy.[a]

	Number of Participants Reporting		
Asbestos, weight-percent[b]	<1.0	1 to 10	>10
Sample No.:			
7154A	0	3	29
7154B	8	17	7
7335	6	15	11
7336	4	22	6
7385	17	0	0
7389	5	0	0
7500	1	0	31
7504	0	3	29
7505	29	2	1
7506	25	7	0
7507	2	16	14
7508	0	2	30
7510	0	0	32
7512	30	2	0

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[a] Laboratory No. 19 results not included.

[b] Value is the sum of all asbestos minerals reported.

On the samples with zero to trace asbestos content such as 7385, 7389, 7505, 7506, and 7512 there was general agreement of <1 percent with one exception. Similarly, for some samples with high asbestos content such as 7154A, 7500, 7504, 7508, and 7510 there was good agreement. In contrast, there were several samples such as 7154B, 7335, 7336, and 7507 where the reported values ranged from zero to high concentrations with a significant number of laboratories in each range. For the latter samples the range of values could reflect either problems in quantification or unacceptable variability in the grab samples submitted to the participating laboratories. Two of these samples, 7154B and 7507, were layered. Sample 7335 had areas of high fiberglass concentration. These types of inhomogeneous samples can pose difficulties to the analyst. Sample 7336 has an asbestos content near 10 percent, so high and low estimates could put it into different categories.

To check the variability of the grab samples, several of the laboratories reporting diverse results were requested to return the unused portion of specific samples to Avondale for homogeneity evaluation. Each of these returned samples was reduced to minus 20 mesh in a Wylie mill, then blended for 4 minutes with a Spex mixer. One-half gram amounts of each sample were prepared as 1-1/2-inch-diameter pellets using a starch-type binder for x-ray diffraction intensity measurements on the chrysotile and amosite peaks.

Sample 7510 had total asbestos concentrations that were fairly consistent among laboratories. However, the reported concentrations for amphibole asbestos and chrysotile ranged from 20 to 95 and 0 to 40 weight-percent, respectively. X-ray diffraction data for five grab samples returned to Avondale by the participating laboratories are listed in Table 3. There is no significant difference in the measured x-ray intensities among the five samples considering the reproducibility of the sample preparation and x-ray measurement. The reader should be cautioned that no attempt was made to achieve high-precision x-ray measurements since this level of precision was not warranted for this study. Similar agreement in x-ray diffraction intensities was obtained on other samples that had large variation in reported concentrations. Therefore, it was concluded that the grab sampling technique employed was adequate for this round robin study.

Table 3. Comparison of PLM Determined Asbestos Concentrations and X-ray Diffraction Intensities - Sample 7510.

Laboratory	Concentration, Weight-Percent Estimates by PLM		X-ray Diffraction Intensity Counts per Second	
	Amphibole Asbestos	Chrysotile	Amosite	Chrysotile
3	75	0	570	320
4	50	5.5	575	365
9	20	5	440	290
12	40	40	480	280
20A	75	25	470	300
B	90	0		
C	95	5		

Two factors that can account for the large variations in reported concentrations are systematic bias by the analysts, and sample heterogeneity at the microscopic level. Systematic bias is defined as the bias imposed by a particular operator's sampling and/or quantification techniques that results in a tendency to have high or low estimates for all samples examined. This bias can be reduced by training and experience using primary or secondary standards of known asbestos content.

Microscopic heterogeneity imposes a serious limitation on the precision, and hence the accuracy, of the concentration measurement. Sample heterogeneity at the microscopic scale is illustrated by the data in Table 4. These data represent visual estimates on six different slides prepared for each sample. At the high concentration levels represented by these samples, there is no serious problem in placing the asbestos concentrations in a trace, minor, or major classification. However, as the asbestos concentration decreases to approximately 1 percent level, the PLM data become increasingly less precise on a relative scale. The data in Table 5 were obtained on blended samples prepared as possible standards for EPA by the Research Triangle Institute. These data are actual point counts by PLM. Samples A, C, H, and K indicate the large relative uncertainty per slide at approximately the 1- to 2-percent level. The 1-weight-percent level is given significance by regulatory agencies in that materials containing 1 percent or more asbestos are to be regulated as asbestos-containing materials [2]. The objective here is to point out the analytical uncertainty at this critical concentration level. This example also points out that at low concentration levels it is possible to have no asbestos present in some slides examined, thus increasing the chances of false negative results. False positive results are the result of misidentification or contamination; both can be eliminated through proper training in sampling and in identification and analytical procedures.

Table 4. PLM Visual Estimate of Asbestos Concentration Using Unmilled Samples.

		Concentration, weight-percent					
Sample	Asbestos Type	Slide No. 1	Slide No. 2	Slide No. 3	Slide No. 4	Slide No. 5	Slide No. 6
2029	Chrysotile	20-30	20-30	30-40	10-20	30-40	40-60
	Amosite	40-50	50-60	60-70	40-50	40-50	30-40
2030	Chrysotile	70-80	50-60	20-30	40-50	40-50	30-40
	Amosite	20-30	30-40	40-60	40-60	30-40	50-60
2031	Amosite	5-20	30-50	5-10	15-25	5-10	15-30
2032	Amosite	8-15	5-15	5-10	5-20	5-10	25-40

Table 5. Variation in Counting Asbestos Fibers Using Polarized Light Microscopy.

	Asbestos Fibers per 50 Particles Counted										
Sample	A	B	C	D	E	F	G	H	I	J	K
Slide:											
1	0	17	0	9	4	0	2	2	10	26	0
2	0	13	1	7	5	0	3	2	26	20	0
3	1	12	0	8	4	0	2	1	19	24	1
4	1	16	0	6	4	ND	3	2	24	30	0
5	1	8	0	9	7	ND	2	0	22	23	0
6	0	11	0	10	2	ND	1	1	25	28	1
7	0	10	0	7	1	ND	1	1	20	27	0
8	1	8	0	12	2	ND	4	0	22	22	0

ND = Not determined.

The Department of Education Asbestos Task Force recently proposed that the critical concentration level for asbestos-containing materials in place be changed from 1 percent to 5 percent [3]. This change would reduce the analytical uncertainty by expanding the <1-percent concentration category, where the analytical estimates are most difficult, to <5 percent. Table 6 shows the roundrobin data categorized using this proposed 5-percent concentration level. With samples 7154B, 7335, 7336, and 7507 excluded for reasons mentioned earlier, there was closer agreement among concentration estimates using <5 percent than with the <1-percent critical concentration level.

Table 6. Quantification of Asbestos Content by PLM Using the 5-Percent Critical Concentration level.[a]

	Number of Participants Reporting	
Asbestos, weight-percent[b] . .	≤5	≥5
Sample No.:		
7154A.	2	30
7154B.	15	17
7335	13	19
7336	17	15
7385	17	0
7389	5	0
7500	1	31
7504	1	31
7505	31	1
7506	31	1
7507	7	25
7508	2	30
7510	0	32
7512	32	0

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[a] Laboratory No. 19 results not included.

[b] Value is the sum of all asbestos minerals reported.

3. Standards for Polarizing Light Microscopy

Based on the round robin and experiences gained in training personnel, two types of standards are required: one for identification training, and another for establishing and monitoring the reliability of the quantitative analyses. The first need would be met by a set of essentially monomineralic standards comprising each of the fibrous minerals and synthetics used in insulation and construction materials. These "pure" standards, both as bulk materials and as prepared slides, would be used to train personnel on the correct identification of asbestos and how to distinguish asbestos from other fibrous materials. The UICC asbestos standards suitable for this purpose are available from Duke Scientific Corporation, 445 Sherman Avenue, Palo Alta, California 94306. This identification training can be greatly assisted by the use of textbooks such as the McCrone Asbestos Particle Atlas [4], as well as basic mineralogically oriented microscopy texts. Short courses of 3- to 5-day duration are also available.

A second group of standards is required for quantification of the asbestos content using PLM. Two suggested approaches are (1) the use of analyzed real world samples for secondary standards and (2) the preparation of primary standards by blending known quantities of materials. The values for selected amphibole and serpentine asbestos in real world samples can be established on a consensus (relative) basis using optical microscopy, x-ray diffraction, and infrared spectrophotometry. However, these techniques are not necessarily absolute because of possible systematic errors; therefore, these secondary standards would be limited in use as relative controls only.

In special cases, absolute values can be obtained from insulation samples or standards. One example of absolute values is the case where the insulation sample is composed of chrysotile plus other constituents that do not lose water of hydration over the same temperature range as chrysotile. With this type of sample, a reliable value of the chrysotile concentration can be obtained by careful measurement of the quantity of released water over a selected temperature range, as measured by weight loss, water absorption, gas chromatography, or mass spectrometry. Because of the moderately high water content of chrysotile, approximately 13.5 weight percent, as compared with the amphiboles' approximately 2 weight percent, quantitative determinations based on measurement of water content are limited to chrysotile except for higher concentrations of amphiboles. Theoretically, the chrysotile content can be determined with an absolute accuracy of ±1 percent using large samples and careful measurement of the analytical signal or the water absorption tubes before and after the controlled heating cycle.

The other approach is to prepare synthetic standards using carefully weighed quantities of the various constituents. Table 7 lists the common components of asbestos insulation materials. The fibrous constituents are asbestos in the form of chrysotile, amosite, or crocidolite; manmade inorganic fibers such as fiberglass and rock wool; and organic fibers such as cellulose and synthetic organics. Wollastonite and fibrous talc are rare components sometimes observed in insulation samples. The nonfibrous components are usually calcite, quartz, talc, vermiculite, mica, clays, gypsum, and perlite. Lime or gypsum mortar is often used as a binding agent.

Table 7. Possible Constituents in Asbestos-Containing Insulation.

Fibrous Constituents	
Amosite	Mineral wool/fiberglass
Chrysotile	Paper fibers
Crocidolite	Synthetic fibers
Nonfibrous Constituents	
Bassanite	Lime
Calcite	Mica
Chlorite	Perlite
Clays	Pumice
Diatoms	Quartz
Ground glass	Talc
Gypsum mortar	Vermiculite

Two major problems with these synthetic standards are the degree of purity of the asbestos minerals and adequate blending of the materials. Although one can accurately weigh the asbestos minerals used in each standard, there is some uncertainty as to their mineralogical purity. As examples, the two chrysotile samples used in the National Institute of Environmental Health Sciences feeding study contained minor amounts of calcite, brucite, talc, quartz, and opaques [5]. The chrysotile content was judged to be >96 percent so that the maximum systematic error due to impurities is less than 5 percent absolute.

The mechanical mixing of the fibrous and platy constituents offers a challenge. A practical method found by the authors was the use of a Wylie mill that consists of a set of rotating knife edges with a small adjustable distance from the knife edges to the inner surface of the mill. The milled particles pass through a screen of selectable mesh size and are collected in a suitable container. Standards prepared by this approach should be suitable for milled unknowns; however, the use of milled standards might be of questionable value for quantification of unmilled samples.

4. Summary

The asbestos minerals in construction materials can be correctly identified using PLM by analysts with appropriate mineralogical education and experience. Round robin participants who incorrectly identified some of the samples would benefit by supervised training using monomineralic standards.

Quantification of the asbestos concentration by PLM is a more difficult problem because of several factors including range of operator experience, lack of suitable standards, and sample heterogeneity at the microscopic level. The reliability of the quantification is particularly serious at the 1- and 5-weight percent concentration levels, decision point concentrations for the regulatory agencies. Improvement in PLM quantification can be anticipated with additional training using primary and secondary standards, and by the use of milled unknowns and standards.

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National Bureau of Standards Special Publication 619. Proceedings of the NBS/EPA Asbestos Standards Workshop held at NBS, Gaithersburg, Maryland, October 1-3, 1980. (Issued March

PREPARATION OF ASBESTOS "STANDARDS" FOR METHODS VERIFICATION AND LABORATORY EVALUATION

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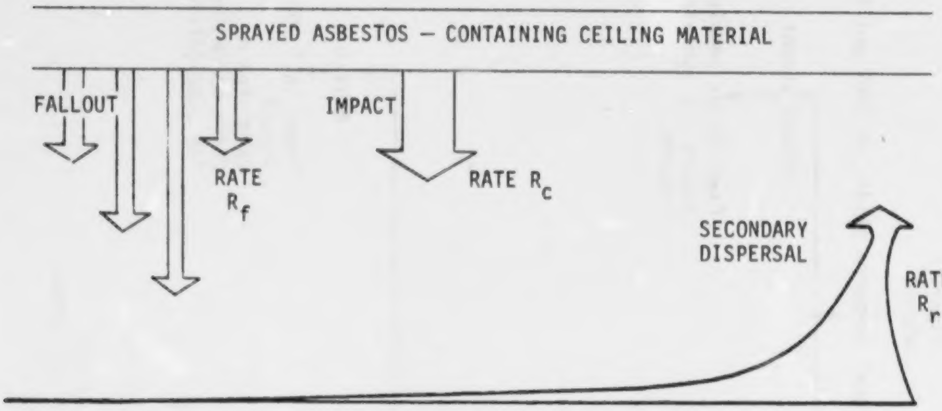
Abstract

The Research Triangle Institute (RTI) has supported the Environmental Protection Agency's "Asbestos in Schools" program by producing a method for the determination of bulk material asbestos content. The utility of the method was investigated in an interlaboratory test program which included formulated samples and samples of in-place sprayed insulation. Preliminary analysis of the results suggests that a reasonable estimate of asbestos weight percents can be made by application of appropriate correction factors to real percent determinations of optical methods. Laboratory evaluation may be best performed through the use of nonparametric ranking of reported results when standard samples are not available.

The potential health hazards of asbestos exposure are now well documented through epidemiological studies of occupational exposures to dusts and fibers. Increased incidence of lung cancers, mesothelioma, and asbestosis are correlated with duration and level of exposure. Presently the Occupational Safety and Health Administration is enforcing standards in work exposure for the 2-3 million workers involved in mining, milling, and production operations involving asbestos. The Bureau of Mines, the Mine Safety and Health Administration, NIEHS, NIOSH, and others are also concerned with various aspects of this same problem.

In addition to the clearly recognized hazards of occupational exposures to asbestos, there is increasing concern over the potential hazards to the general population of exposures resulting from release of asbestos into the environment from widely distributed asbestos-containing materials. At present, over 850,000 tons of asbestos are used in the U.S. each year, a significant percentage of which is eventually released into the environment. Preliminary attempts have been made to assess the levels of such exposures and the associated health hazards; however, further investigation is needed.

The Environmental Protection Agency has a clear responsibility both in the area of health risk assessment and in control strategy development. As a part of the Agency's effort to meet this responsibility, initial attention was focused on the problem of asbestos exposures in schools resulting primarily from release of asbestos from friable insulating materials (figure 1). For the past two years, RTI has been involved in providing technical assistance to the EPA in this program, including both the evaluation of laboratories performing asbestos analysis and the development of reliable methods for quantitative analysis. As a part of this work, it was necessary to develop well-characterized asbestos-containing samples for validation of the proposed method. This presentation will focus on the use of "standard" samples in evaluating analytical methodologies and laboratory performance. The word "standard" is qualified to the extent that final values for the weight percent of asbestos in each sample series were calculated using mean values for matrix weight loss due to drying and dissolution during sample preparation.



MODE	CAUSES	FREQUENCY	RATE
FALLOUT	AIR MOVEMENT VIBRATION	CONSTANT	LOW (R_f)
IMPACT	MAINTENANCE ACCIDENTAL IMPACT	OCCASIONAL	HIGH (R_c)
SECONDARY DISPERSAL	USUAL ACTIVITY CUSTODIAL SERVICE	FREQUENT	LOW TO HIGH (R_r)

Figure 1. Modes and rates of fiber dispersal. (From "Sprayed Asbestos-Containing Materials in Buildings", EPA-450/2-78-014.)

A method was developed for the analysis of asbestos in bulk insulation material by polarized light microscopy (PLM) as shown in figure 2. An x-ray powder diffraction (XRD) protocol was included as an auxiliary method to be used when independent confirmatory analysis is required. Procedures for qualitative identification and quantitation were included for both techniques; quantitation in the PLM protocol is performed by point counting. This method was carefully drafted from available source information and was based in large part on information obtained in an RTI coordinated symposium at the Bureau of Mines Avondale Research Center, October 23-25, 1979 for the EPA Office of Pesticides and Toxic Substances. Symposium participants and several commercial laboratories reviewed the method prior to validation testing.

In an effort to validate the proposed method, an interlaboratory testing program was designed and executed with the following objectives:

1. Evaluate the inter-laboratory precision and accuracy and intra-laboratory variation in applying this interim method;

2. Compare the resulting measure of precision with that obtained in other studies using different PLM protocols; and

3. Evaluate the error rate of the method relative to the federal 1-percent weight criterion for asbestos content of sprayed-on insulation materials (38 FR 8830; April 6, 1973).

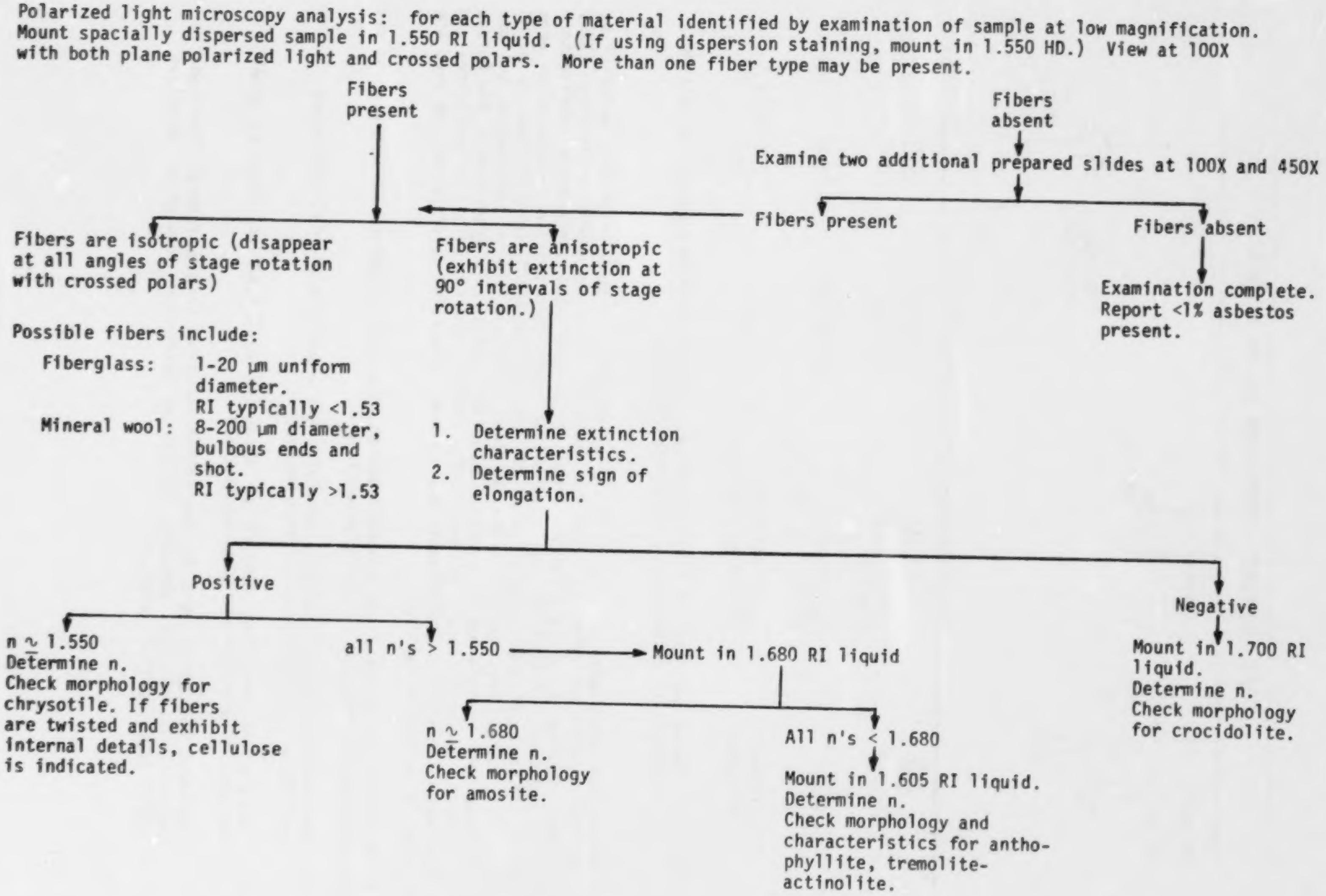

Polarized light microscopy analysis: for each type of material identified by examination of sample at low magnification. Mount spacially dispersed sample in 1.550 RI liquid. (If using dispersion staining, mount in 1.550 HD.) View at 100X with both plane polarized light and crossed polars. More than one fiber type may be present.
Fibers present
Fibers absent
Examine two additional prepared slides at 100X and 450X
Fibers present
Fibers absent
Examination complete. Report <1% asbestos present.
Fibers are isotropic (disappear at all angles of stage rotation with crossed polars)
Possible fibers include:
Fiberglass: 1-20 µm uniform diameter. RI typically <1.53
Mineral wool: 8-200 µm diameter, bulbous ends and shot. RI typically >1.53
Fibers are anisotropic (exhibit extinction at 90° intervals of stage rotation.)
1. Determine extinction characteristics.
2. Determine sign of elongation.
Positive
Negative
n ~ 1.550 Determine n. Check morphology for chrysotile. If fibers are twisted and exhibit internal details, cellulose is indicated.
all n's > 1.550
Mount in 1.680 RI liquid
n ~ 1.680 Determine n. Check morphology for amosite.
All n's < 1.680
Mount in 1.605 RI liquid. Determine n. Check morphology and characteristics for anthophyllite, tremolite-actinolite.
Mount in 1.700 RI liquid. Determine n. Check morphology for crocidolite.

Figure 2. Flow chart for analysis of bulk samples by polarized light microscopy.

Adequate evaluation of the proposed method required the use of well-characterized samples. Factors to be considered were:

1. asbestos type,
2. representativeness as compared to real-world samples,
3. homogeneity,
4. matrix interferences,
5. degree of characterization obtainable, and
6. end use of data.

When the use of actual ceiling insulations was considered for the evaluative studies, it was clear that there were major difficulties in the areas of homogeneity, matrix interferences, and degree of characterization obtainable. As a result, a decision was made to formulate a series of samples containing known amounts of asbestos (either chrysotile or amosite) in a relatively simple matrix material.

Eight series of samples, targeted at specific weight-percents of asbestos fiber, were formulated. Table 1 presents target weights and allowable limits for matrix material and asbestos fiber in each series. The formulated samples contained a single matrix to assure comparability within and hopefully between asbestos types. The actual weight percent of asbestos in each sample series was determined after applying a correction factor for weight loss due to dissolution of part of the matrix in the mixing step.

Table 1. Target Weights and Allowable Limits for Matrix Material and Asbestos Fibers.

Series	Target wt %	Fiber Type	Weight of Asbestos (g)	Weight of Matrix (g)
C	1	Chrysotile	0.05 ± 0.005	4.95 ± 0.05
A	4	Chrysotile	0.2 ± 0.01	4.80 ± 0.05
E	16	Chrysotile	0.8 ± 0.01	4.20 ± 0.05
I	64	Chrysotile	3.2 ± 0.01	1.80 ± 0.05
H	2	Amosite	0.1 ± 0.01	4.90 ± 0.05
G	8	Amosite	0.4 ± 0.01	4.60 ± 0.05
D	16	Amosite	0.8 ± 0.01	4.20 ± 0.05
B	32	Amosite	1.6 ± 0.01	3.40 ± 0.05

Blanks were provided as controls and for determining the method's potential for producing false positives. A "real world" sample containing approximately 50 percent chrysotile was included for comparison to determine whether precision estimates from formulated samples are misleading. Duplicates were included without the laboratories' prior knowledge to estimate within-laboratory variance.

The sample components were carefully weighed for each sample. The asbestos was suspended in a 0.05 percent sodium dodecyl sulfate solution and sonicated to ensure good separation of the mill grade asbestos. This suspension was mixed with the predominantly gypsum matrix in a blendor, vacuum filtered and oven dried. Quality control procedures employed included microscopic examination and duplicate weighings.

Twenty laboratories returned PLM results on the sample sets. There were several false negatives at the target one percent loading and one false negative at the target four percent loading. No false negatives were reported for samples containing amosite or more than five percent chrysotile. There was one false positive.

Six laboratories returned XRD results; three of these performed the requested analyses using some variation of the proposed thin-layer method of quantitation; three used alternative bulk or thick-layer methods of quantitation. However, none of the methods were strictly equivalent.

The following statements are preliminary observations which are subject to revision pending further evaluation.

(1) The point count method appears to be a reasonable but somewhat high estimator of asbestos content.

(2) Laboratories using the point count procedure reported more accurate results than those laboratories using their own quantitation procedures.

(3) XRD results were generally good, and of comparable accuracy and precision to the point-count method.

Because the result produced by XRD quantitation is directly related to the amount of material present, calculation of precision and accuracy for the XRD evaluation is straightforward. In the PLM evaluation, however, area is measured and then related to weight. This theoretically necessitates an adjustment of the PLM data before reporting the analysis. The following geometric and physical considerations apply.

1. Weight W is proportional to volume V;
2. V is proportional to the cube of linear measure L;
3. Area is proportional to the square of linear measure L.

Therefore, an anticipated model for the area-weight relation is the power law, $A = bw^c$ or $\log(A) = c \log(W) + \log(b)$, with $c = 2/3$.

Reported PLM results are area proportion estimates (A), which require numerical adjustment in order to be regarded as weights (W). If data from all point count laboratories are pooled and the model is fit with $c = 2/3$ and b allowed to vary with asbestos type, then the Pearson correlation coefficient r equals .91.

If laboratories are individually calibrated, so that the constant b is allowed to vary with asbestos type and laboratory, then r equals .98.

After fitting the model to the PLM data, empirical values for the asbestos-type-dependent parameter b, with $c = 2/3$ are used to transform the reported areas to weight percent values. Precision and accuracy are then determined by comparison of the derived weight estimates with the actual weight loadings.

Although the areas reported by PLM cannot be reasonably regarded as weights, and the area vs. weight calibration changes with asbestos type and matrix, area values as reported seem to carry considerable information, in that they tend to preserve the correct order of the asbestos concentration. That is, if one sample has a greater weight proportion of asbestos than another, then the PLM reported area will generally be greater. In this study, it appeared to be the case without adjustments for asbestos type.

A statistical approach which utilizes this information involves the use of a correlation coefficient. The ordinary, or "Pearson" correlation coefficient is a measure of the association between two variables, x and y (figure 3). In the method evaluation discussed above, x was the loaded weight and y was the transformed area data. The value of r may vary between negative one and positive one, a value of zero indicating no relationship between the variables.

- $r(x,y)$ is a measure of the association between two variables, x and y
- $r(x,y) = [1/n \sum_{i=1}^{n} (x_i - \overline{x})(y_i - \overline{y})]/[(S_x)(S_y)]$
- $-1 \leq r \leq 1$
- If x and y are unrelated, then $r = 0$
- If x and y are linearly related, then $|r| = 1$

Figure 3. Pearson correlation coefficient $r(x,y)$.

The Spearman rank correlation coefficient (r_s) is a non-parametric measure of the relationship between two variables. It may be computed by replacing area and weight values by their relative ranks, and then computing the ordinary (Pearson) correlation between the pairs of ranks (figure 4).

- r_s is a nonparametric measure of the association between two variables, x and y
- Replace x_i and y_i by their within-group ranks $R(x_i)$ and $R(y_i)$;
- $-1 \leq r_s \leq 1$
- If x and y are unrelated, then $r_s = 0$
- If x's and y's are in same relative order, then $|r_s| = 1$

Figure 4. Spearman rank correlation coefficient $r_s(x,y)$.

This approach is applied to results reported by a laboratory in the method evaluation study for illustration (Table 2). For samples containing amosite, the loaded weight (X), and the reported area (Y), are each ranked in ascending order. The ranks match exactly, and r_s equals 1 (see Table 2). If two ranks are reversed, r_s equals 0.9. If three ranks are misplaced, r_s equals 0.7.

Table 2. Spearman Rank Correlation within Laboratory.

(i) Amosite results of Lab #7.
$r_s = 1$

R(x)	Loaded Weight (%) x	Reported Area (%) y	R(y)
5	35.6	63	5
4	17.8	48	4
3	9.0	36	3
2	2.3	17	2
1	0.0	0	1

(ii) With positions reversed,

$r_s = 0.9$		$r_s = 0.7$	
R(x)	R(y)	R(x)	R(y)
5	4	5	3
4	5	4	5
3	3	3	4
2	2	2	2
1	1	1	1

In our preliminary results on the method validation study we find that, with no calibration or correction for asbestos type, the average Spearman correlation coefficient (r_s) between weights and reported areas (not transformed) is 0.91. This compares favorably with the Pearson coefficient (r) of 0.92 obtained by transforming data and calibrating chrysotile separately from amosite. That is, if area ranks (unadjusted for asbestos type) are analyzed, then the corresponding correlation is as high as is obtained by analyzing the original data separately for each asbestos type.

A rank based approach may also be used to check whether a laboratory tends to report excessively high or low results. This involves the same type of analysis applied across laboratories. For example, in figure 5, laboratory H (1 of 20 laboratories in the point count group) was ranked either first or second on all samples. The chances of a result this extreme occurring by chance alone is less than one in a million. Clearly laboratory H produces outlying values. (They are, however, internally consistent [Spearman correlation = 0.95].)

Another variation on this approach may be used to test for significant within-laboratory imprecision.

For the past year a quality assurance program has been conducted for laboratories which analyze bulk materials for asbestos using mill-grade materials. The purpose of the program is two-fold: first, to provide laboratories with simple characterized samples for quality control; second, to preliminarily evaluate laboratory performance on qualitative and quantitative analysis of bulk samples for asbestos. Over 130 commercial and non-commercial laboratories have participated. Quarterly mailings of 4-6 samples per laboratory are planned

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    !        F       S              D
    !    D   E       P              H
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    !  J                            H
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    !  K H   G
0.0 +D G     H
    -+-------+-------+-------+-------+-------+-------+-------+-------+-------+-------+-------+-------+-------+-------+-------+---
   0.00    0.05    0.10    0.15    0.20    0.25    0.30    0.35    0.40    0.45    0.50    0.55    0.60    0.65    0.70    0.75
                                                                 WEIGHT
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Figure 5. Statistical analysis system. Group=P. Plot of A*W. Symbol is value of Laboratory.

for the coming year. This will require >2500 samples to be distributed over the next year. Preparation of each of these samples in the RTI laboratory would not be cost effective. Neither would it allow adequate representation of the range of sample conditions which occur in in-place sprayed materials.

Future interlaboratory studies will use samples taken from in-place sprayed-on insulation since individual sample preparation and statistical analysis is not always possible or warranted. Bulk materials for these samples will be screened by RTI for within-batch variability, and distributed to participating laboratories for analysis. Each laboratory results will then be compared with the means of all laboratories results to evaluate within-laboratory consistency. Laboratories may be tested for extreme bias by looking at within-sample ranks to see if the laboratory is consistently high or low. Finally, laboratories may be evaluated with respect to precision by a related non-parametric rank-based procedure as illustrated in this paper. This approach reduces the burden of assuring that each sample is individually characterized and less information on each sample is required. Laboratory reports nonetheless yield information with which to evaluate their respective performance. Larger sample distribution programs for the purpose of evaluating laboratory performance are therefore feasible.

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SAMPLE PREPARATION FOR QUANTITATIVE ELECTRON MICROSCOPE ANALYSIS OF ASBESTOS FIBER CONCENTRATIONS IN AIR

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Abstract

The ability to determine asbestos fiber concentrations and size distributions depends on the electron microscope observation of the particles in very small fields of view that constitute microcosms representative of the total sample. Sample preparation procedures are a critical consideration in the quest for acceptable accuracy and precision because they have a direct influence on the number and size of particles observed. Loss of particles, physical or chemical degradation, particle contamination, alteration of particle size distribution, interference by debris, and non-uniform particle distribution are problems associated with sample preparation procedures. Basic methods for collecting particles suspended in air or water on membrane filters and then transferring them to electron microscope grids will be discussed with respect to these problems. Preparations of biological samples (tissues, food, fluids) and standard samples from dry powders are complicated by the need to manipulate the sample to obtain particle suspensions in water suitable for filtration on a membrane filter. The desirable effects of the use of ashing, chemical digestion, surfactants, sonification, centrifugation, and agitation techniques must be weighed against the possibilities for sample alteration associated with each manipulation of the sample. Results from interlaboratory comparison studies have indicated that different sample preparation procedures frequently result in poor agreement between the laboratories using the procedures.

1. Introduction

In the past decade electron microscopic techniques for identifying and counting asbestos particles in water and air have been extensively developed and applied. The most useful exposure data for evaluating health risks associated with non-occupational exposures to asbestos and analogous mineral and synthetic fibers is fiber concentration and size distribution determined by electron microscopy. The identification techniques of morphology, selected area electron diffraction and energy dispersive x-ray spectroscopy available for transmission electron microscopy (TEM) are essential for the discrimination of asbestos fibers from organic or non-asbestos mineral particles with aspect ratios exceeding 3:1.

The ability to determine asbestos fiber concentrations and size distributions depends on the electron microscopic observation of the particles in very small fields of view that constitute microcosms representative of the total sample. Sample preparation procedures are a critical consideration in the quest for optimum accuracy and precision because they have a direct influence on the number and size of particles observed. Loss of particles, physical or chemical degradation, particle contamination, alteration of size distribution, interference by debris, and non-uniform particle distribution are problems associated with sample preparation procedures.

Although preparation procedures for air, water, tissue and other types of samples incorporate common steps and principles, the sample type (e.g., water, air, tissue, sediment) may influence the behavior of particles during sample preparation. For example, water filtration of particles onto a membrane filter produces a more stable deposit than that received from filtration of suspended particles in air. This difference seems to cause more extensive particle loss or rearrangement for air samples than for water samples during direct transfer of uncoated filter pieces to TEM grids. With these sample related differences in mind, this discussion of the influence of sample preparation procedures on asbestos fiber analysis will concentrate on methods for asbestos fibers in air. An excellent comprehensive review of fiber counting techniques for air and water samples, including sample preparation procedures, has been prepared by Chatfield [1][1].

2. Basic Procedures for Transfer of Particles from Membrane Filters to TEM Grids

Most air analysis techniques in use involve filtration of particles from the air with either a Millipore or Nuclepore membrane filter[2]. The estimation of how much air to filter has an important impact on sample preparation. The loading of particles on the filter must be controlled to ensure adequate sensitivity without accumulating so much sample that particles are obscured by other particles on the TEM grids. Sample preparation procedures can be chosen to either increase or decrease the number of particles per unit area on the TEM grid in comparison to the original filter loading. This requires resuspension and refiltration of the particles removed from the original membrane filter.

Particles on the surface of membrane filters can be transferred directly to a carbon-coated TEM grid by solvent dissolution of the filter (figure 1a). Modified Jaffe washer [2] techniques or condensation washers [3] are frequently used to dissolve the filter. The extremely flat surface of the Nuclepore membrane filter allows a thin carbon film (approximately 50 nm) to be applied with a vacuum evaporator so that particles on the filter surface are embedded in the carbon film. Filter pieces placed on TEM grids can be dissolved in chloroform with a Jaffe wick technique (figure 1b) to leave the carbon film with particles embedded in it on the TEM grid [4]. This retention of particles in a carbon film prevents movement and loss of particles during solvent dissolution of the filter. Other membrane filters such as the Millipore type do not produce satisfactory carbon replicas because of their very rough surface texture. Unfortunately, direct transfer preparation of TEM grids from air particulates on uncoated membrane filters with rough surfaces can result in greater than 90 percent loss of the particles, especially when the filter is heavily loaded. The EPA provisional method for electron microscopic measurement of airborne asbestos concentrations [5] utilizes the carbon coated Nuclepore filter direct transfer technique.

Brief exposure of Millipore filters to acetone vapor results in the reduction of the rough surface and sponge-like inner filter structure to a continuous film with most particles on the surface [6]. This allows carbon coating of the filter and TEM grid preparation in the same way as previously described for carbon coated Nuclepore filters (figure 1b) except that acetone is used as the solvent. This "collapsed membrane filter" method is used by NIOSH for air samples [7]. Some fibers may be lost if they are captured in the interstices of the filter rather than on the surface where they can be embedded in the carbon film. The acetone vapor treatment of filters prior to transport from the field would prevent loss of particles from the filter surface prior to the TEM grid preparation in the laboratory.

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[1] Figures in brackets indicate the literature references at the end of this paper.

[2] Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. Environmental Protection Agency.

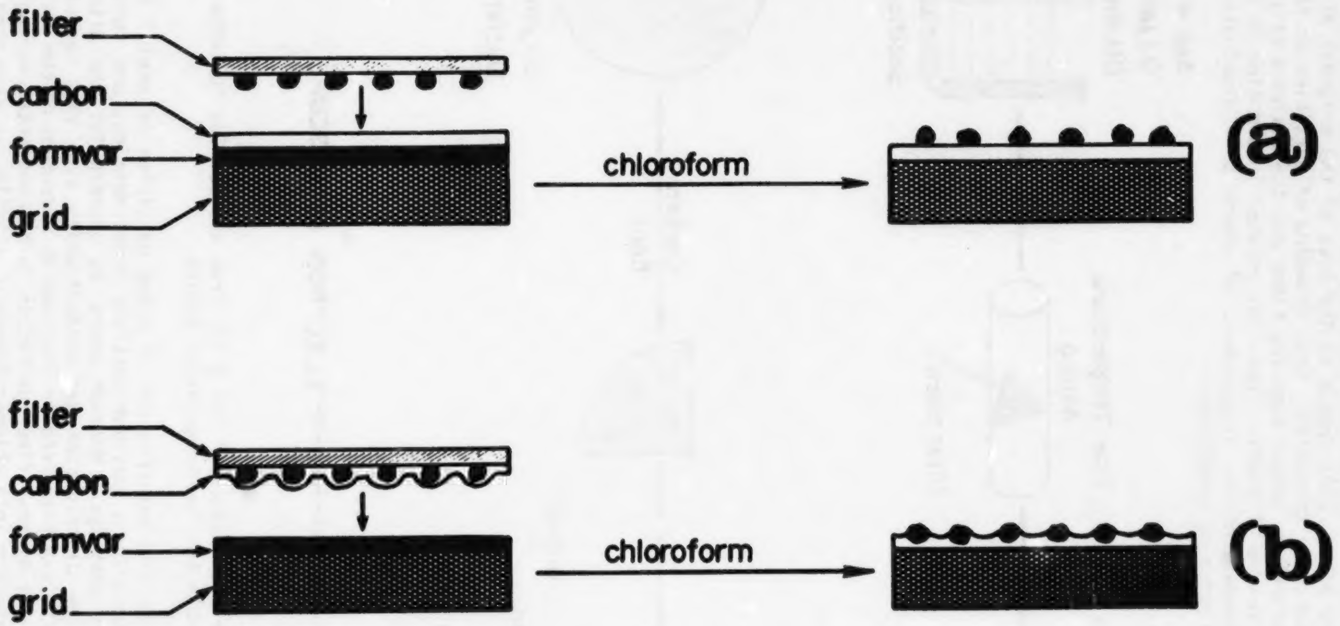
filter
carbon
formvar
grid
chloroform
(a)
filter
carbon
formvar
grid
chloroform
(b)

Figure 1. Preparation of electron microscope grids by direct transfer of particles from a Nuclepore filter. (a) Filter to carbon-coated grid. (b) Carbon-coated filter to grid.

A technique used at this laboratory for preparing TEM grids for particulates collected from large volumes of air on 0.45, 0.8 or 1.2 micrometer pore size Millipore filters is illustrated in figure 2. The ratio of the Nuclepore filter area used for filtration of resuspended low temperature ashed (LTA) sample to the area of the original Millipore filter ashed typically ranges from five to twelve. Less crowding of particles on the TEM grid is thus achieved. This method allows longer sampling times and the analysis of average samples by combining pieces from several filters. Thus, far greater information is obtained about average human exposure concentrations independent of short term fluctuations caused by varying meteorological and emission conditions.

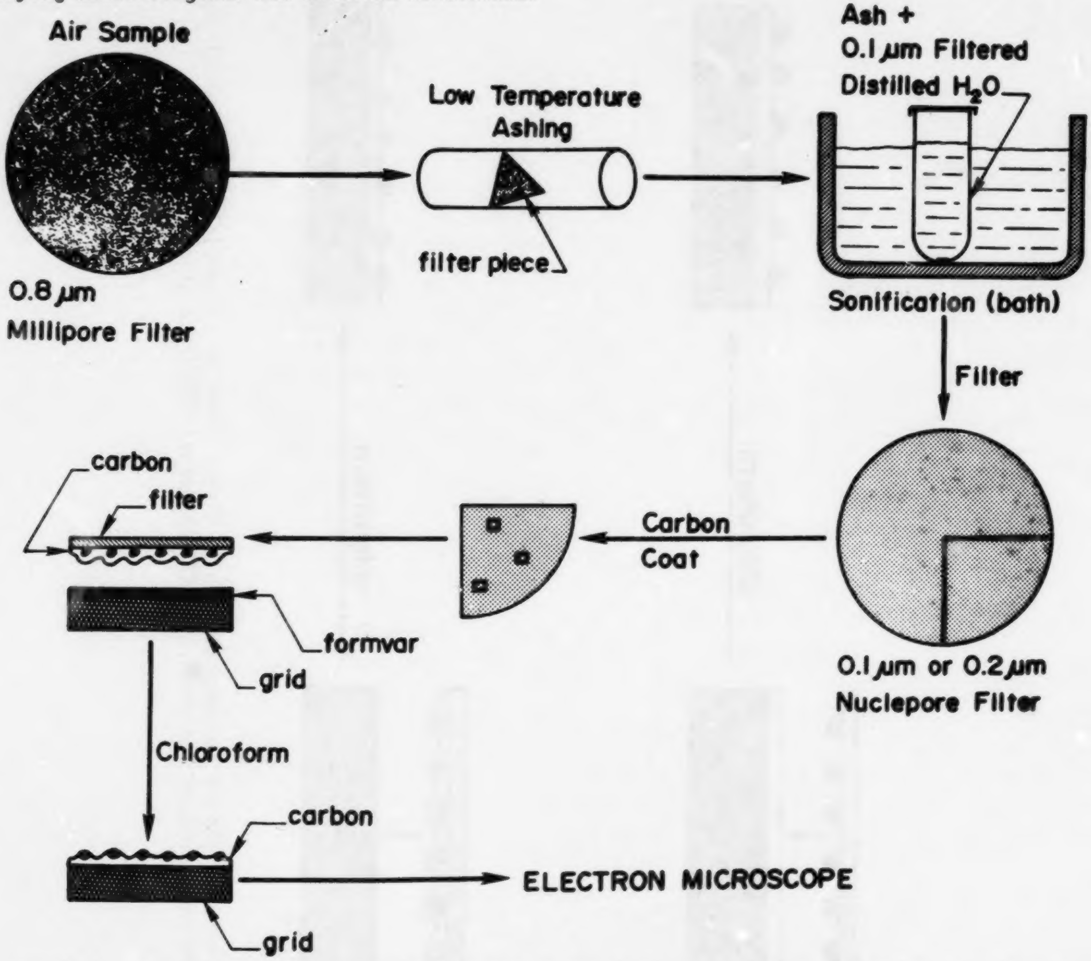
Air Sample
0.8 µm
Millipore Filter
Low Temperature
Ashing
filter piece
Ash +
0.1 µm Filtered
Distilled H2O
Sonification (bath)
Filter
0.1 µm or 0.2 µm
Nuclepore Filter
Carbon
Coat
carbon
filter
formvar
grid
Chloroform
carbon
ELECTRON MICROSCOPE
grid

Figure 2. Technique for preparation of TEM grids from air samples collected on membrane filters suitable for low temperature ashing.

Low temperature ashing and resuspension of ashed particles is useful for insuring a uniform distribution of particles, optimum particle areal density, and removal of interfering organic debris and coatings. However there is concern for the alteration of the original mineral fiber size distribution and concentration with this technique. This is most likely to happen in the ash resuspension step and is dependent on the particular procedures used. The tendency for mineral fiber particles to be comminuted during ash resuspension, particularly by separating into thinner fibers or fibrils, is also greatly influenced by particle mineralogy. Samples containing amphibole fibers on Nuclepore filters have been prepared by both the direct carbon-coated filter technique and the LTA/resuspension technique described in figure 2. The TEM grids produced differed only in the sample preparation technique used (the resuspended samples were neither concentrated nor diluted).

Amphibole fiber concentrations determined for the two preparation techniques indicate a tendency for underestimation (up to 30%) of amphibole fibers following LTA and resuspension with low energy ultrasonic energy. This may result from failure to separate some ash particle aggregates so that some amphibole fibers are not identified or from the loss of some particles during low temperature ashing.

Chrysotile fibers are potentially far more susceptible to comminution during low temperature ashing and resuspension because of the mineral's fibrilar structure and relatively weak interfibril bonding. Different chrysotile mineralizations produce variations in chrysotile fiber properties which create variable resistance to ultrasonic energy and other resuspension techniques. The use of surfactants such as aerosol OT for resuspension of chrysotile particles dramatically reduces the mineral to individual fibrils. Chrysotile often occurs in air samples as bundles, clumps, and loose mats of fibrils in addition to single fibrils. It is unlikely that any LTA and resuspension procedure can prevent the dislocation of some fibrils making up these complex aggregates. The increase in number of fibers and fibrils counted following LTA and resuspension can be minimized, however, by using the least ultrasonic energy necessary to achieve a uniform distribution of particles on the TEM grids.

An outline of options used for air sample preparation is provided in Table 1. Besides the previously discussed direct techniques and LTA variations, other techniques involving resuspension of particles prior to grid preparation are listed. High temperature ashing is not recommended because severe clumping of particles can occur as well as alteration of asbestos fibers. Chrysotile, for example, begins to undergo thermal decomposition at approximately 500 °C [8]. The micropipette method of preparing TEM grids by drying a drop containing resuspended particles on a carbon coated grid [9] is also not recommended due to the difficulty in consistently achieving a uniform distribution of particles.

Table 1. Techniques for Air Particulate Transfer from Membrane Filters to TEM Grids: An Outline.

I. Direct Preparation from Filter
   A. Nuclepore Filter
      1. Without carbon coat/Jaffe wick
      2. With carbon coat/Jaffe wick
   B. Millipore Filter
      1. Condensation washer
      2. Without carbon coat/Jaffe wick
      3. With carbon coat/Jaffe wick
      4. Collapsed filter with carbon coat/Jaffe wick

II. Ashing
   A. High Temperature/Resuspension/Filtration/Direct Transfer
   B. Low Temperature/Resuspension/Filtration/Direct Transfer
   C. Low Temperature/Resuspension/Micropipette (drop on carbon coated grid)
   D. Low Temperature/Rubout

III. Solvent Dissolution of Filter/Rususpension/Filtration with Nuclepore/Direct Transfer

The rubout technique [10] involves grinding the low temperature ashed sample on a slide with the edge of a watch glass and embedding the dispersed particles in a nitrocellulose film. Portions of the film are mounted on TEM grids. The purpose of the rubout technique is to reduce fiber bundles to individual fibers (fibrils in the case of chrysotile) so that a fiber mass concentration can be more precisely estimated. Since the original sample size distribution is radically changed for chrysotile, the rubout technique cannot be used to determine the original fiber number concentration. The same problem exists when extensive, high energy ultrasound treatment of ashed air samples is used, often with a surfactant, to eliminate fiber bundles and clumps.

The dissolution of the membrane filter or a piece of the membrane filter in a solvent which can then be refiltered through a Nuclepore filter has been suggested as a method which would avoid ultrasonic resuspension of particles. Aside from the problem of finding a reliable solvent for completely dissolving the filter and allowing a clean refiltration, it is difficult to ensure that the resulting membrane filter will have a uniform deposit of particles without the use of ultrasound prior to refiltration. Little or no data exists for the adequacy of other agitation techniques (shaking, swirling) as a substitute for ultrasonic treatment.

A clean room facility and/or a laminar flow hood to provide a filtered air environment for sample preparation is necessary to minimize sample contamination particularly with chrysotile fibers. The worst contamination threat may be from improperly cleaned glassware or unfiltered solvents. Membrane filters themselves contain measurable levels of chrysotile fibers [11] which must be considered when ashing the filter. If a large piece of membrane filter is ashed and refiltered on a much smaller area filter, the number of chrysotile fibers attributable to the filter can become unacceptable. Blank samples which reflect all sources of contamination are essential for each preparation of grids.

3. Interlaboratory Comparisons as Influenced by Sample Preparation Technique

Several sets of air samples have been collected in recent years and analyzed by different laboratories with wide variation in fiber concentrations reported. Each laboratory used basically the same fiber identification and counting criteria. Personnel or electron microscope capability differences do not explain the large systematic differences found between laboratories. The different sample preparation procedures used seem to explain all or most of the interlaboratory disagreement.

3.1 Amphibole Fibers

Table 2 provides amphibole fiber concentrations reported by six laboratories for a series of 12 air samples collected by the Minnesota Department of Health in 1975. Each laboratory received a piece of 1.2 µm pore size Millipore membrane filter to analyze for each air sample. Particulate collection on the filters was accomplished with Hi-Vol air samplers operating for approximately 55 hours so that visibly heavy sample loadings were achieved (approximately 5 cubic meters of air per square centimeter of filter). Because small amphibole fibers such as those in these air samples are not easily comminuted, they serve as good indicators of fiber concentration underestimates introduced by fiber loss during sample preparation or fiber obscuration by debris or overlapping particles.

In addition to the amphibole fiber concentration reported for different sample preparation techniques used by each laboratory, Table 2 contains x-ray diffraction measurements of total amphibole mineral mass concentration on the filters. The heavy loading of particles on the membrane filters made them suitable for direct x-ray diffraction analysis and provided an independent measurement to which the amphibole fiber concentrations can be compared [12]. Similar amphibole fiber size distributions and ratios of amphibole fibers to total amphibole particles are indicated by electron microscopic measurements for the 12 samples, so amphibole fiber concentrations should correlate with the x-ray diffraction measurements of amphibole mass.

The three laboratories using LTA followed by different TEM grid preparation steps reported fiber concentrations averaging thirty or one hundred times greater than fiber concentrations obtained from samples prepared by the two laboratories using direct grid

Table 2. Amphibole Fiber Concentrations (10^3 fibers/m^3) for Air Samples Collected on 1.2 μm Millipore Filters: Six Laboratories Using Different Grid Preparation Techniques.

Sample Number	Amphibole Conc. (μg/m^3) X-ray Diffraction	LTA/Rubout	LTA/C-Coat Nuc-Jaffe	LTA/Drop	Direct-CW	Direct-Jaffe	C-Coat Direct/Jaffe
7144A	4.08	335	262	390	5.9	5.5	99
7144B	2.64	164	235	177	2.7	5.4	110
7144C	2.34	323	178	174	3.0	6.6	91
9040	8.74	384	513	450	3.9	12.8	100
9041	8.89	502	448	351	2.5	6.1	160
9042	9.82	583	516	569	0.8	6.2	291
9061	1.66	53	33	67	1.0	1.6	74
9062	3.05	358	71	112	5.8	12.4	215
9063	3.19	240	76	120	0.6	3.8	20
4221	3.73	252	158	138	4.4	10.4	50
4222	2.28	100	99	96	1.4	8.0	70
4223	4.3	394	230	221	3.2	20.6	84
Average		307	230	239	2.9	8.3	114

preparation techniques. One laboratory used a condensation washer and the other laboratory the Jaffe wick method to dissolve away pieces of Millipore filter placed on carbon coated TEM grids. These great differences in results have been attributed by Peters and Doerfler [13] to an increase in the number of fibers in the LTA/resuspension techniques due to fracturing of large amphibole fibers into many smaller fibers. They conducted comparisons of uncoated Millipore/direct and LTA/ultrasonic resuspension techniques for air samples from the same set (Table 2) and concluded that the fiber size distributions for resuspended samples were significantly shifted to smaller sizes, proving that fiber comminution had occurred. We disagree with these conclusions for the following reasons:

1. Ultrasound even of long duration and high energy is not of sufficient energy to cause amphibole fiber cleavage.
2. Although the percentage of large fibers measured in resuspended samples was less than that measured in direct preparation samples, the number of fibers in every size category was far greater for resuspended samples.
3. Simple carbon coating of the Millipore filters followed by direct transfer to TEM grids produced fiber concentration estimates averaging fifteen to forty times the concentration estimates from direct preparation of uncoated filters (Table 2) despite the severe crowding of particles and rough carbon replica of the 1.2 μm Millipore filter surface. In our opinion the carbon film embedding the particles prevents the loss of particles during grid preparation.
4. Our own attempts to prepare grids from these and other air samples by the direct Jaffe wick transfer of filter pieces without carbon coating produces grids with non-uniform particle distributions radically lower in particle numbers than expected from optical microscope examination of the original filter.

5. Particle loss during grid preparation should occur preferentially for smaller particles on uncoated filter pieces.

6. Samples prepared by LTA followed by resuspension were filtered so that particle density on the filter was much lower than on the original filter. This allows easier identification of particles, particularly small fibers.

Additional comparisons are available in Table 3 which contains linear regression analysis correlation coefficients for comparison of each set of results from the six different sample preparation methods. Significant correlations at the 99.5 percent confidence level were found between each of the LTA/resuspension methods and also the x-ray diffraction results. The two direct uncoated Millipore techniques produced data with no correlation to each other, any of the other data sets, or the x-ray diffraction results. The carbon-coated Millipore direct technique produced data which correlated with the LTA/resuspension and x-ray diffraction data at the 95 percent confidence level. These correlations provide powerful evidence for our observation that direct transfer of air samples without carbon coating results in large and variable loss of particles and that gentle resuspension techniques do not result in alteration of the original amphibole fiber size distribution or number concentration.

Table 3. Interlaboratory Comparison of 12 Air Samples: Amphibole Fiber Analysis Correlation Coefficients.

	X-ray Diffraction	LTA/Rubout	LTA/C-Coat Nuc-Jaffe	LTA/Drop	Direct-CW	Direct-Jaffe	C-Coat/Direct-Jaffe
X-ray Diffraction	1.00	0.33[a]	0.94[a]	0.89[a]	0.06	0.15	0.59
LTA/Rubout	0.83[a]	1.00	0.77[a]	0.82[a]	0.18	0.33	0.69
LTA/C-Coat Nuc-Jaffe	0.94[a]	0.77[a]	1.00	0.95[a]	0.01	0.15	0.52
LTA/Drop	0.89[a]	0.82[a]	0.93[a]	1.00	0.07	0.05	0.59
Direct-CW	0.06	0.18	0.01	0.07	1.00	0.43	0.06
Direct-Jaffe	0.15	0.33	0.15	0.05	0.43	1.00	0.04
C-Coat/Direct-Jaffe	0.59	0.69	0.52	0.59	0.06	0.04	1.00

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[a] Significant correlation at 99.5% confidence level.

Portions of an air sample collected on a 1.0 μm pore size Nuclepore filter for amphibole fiber analysis have been prepared by the carbon coated Nuclepore Jaffe wick technique and the LTA/ultrasonic resuspension/carbon coated Nuclepore Jaffe wick technique [14]. No significant difference existed for amphibole fiber size distribution or fiber concentration for the two preparation techniques. This direct comparison of the two sample preparation techniques provides conclusive evidence for the efficacy of the LTA and resuspension technique.

3.2 Chrysotile Fibers

Table 4 provides chrysotile fiber concentrations reported by seven different laboratories for a series of nine air samples provided by the EPA Environmental Monitoring and Support Laboratory, Research Triangle Park, NC in 1977 [15]. Samples were collected on 102 mm diameter 0.45 μm pore size Millipore membrane filters with membrane samplers located near a gravel road covered with crushed serpentine rock containing chrysotile. The dust generated from this rock contains chrysotile [16] in a great variety of forms including small fibrils, fibers, large bundles and clumps, and chrysotile locked in a matrix of antigorite. Most of the chrysotile mass was present in particles other than a single chrysotile fibers or bundles. Filter loading achieved was heavy (1.3 cubic meters of air per square centimeter of filter). Some sample loss appeared to occur during shipment of the filters in glycine envelopes and uneven distribution of particulate was observed on the filters probably due to uneven air flow through the filter during sample collection.

Chrysotile fiber concentrations reported for each sample range over four orders of magnitude. When sample preparation techniques used are considered, these results are not surprising. Very large fiber concentrations were obtained only for those sample preparations which utilized harsh resuspension steps such as grinding (rubout), high energy ultrasound, or surfactant treatment plus ultrasound. These techniques not only separated chrysotile fibers from fiber bundles and clumps, but probably caused the release of chrysotile fibers from chrysotile originally locked in a matrix of non-fibrous minerals. Evidence for the latter effect is provided by each laboratory's estimate of chrysotile fiber mass concentration: the three preparation techniques using harsh resuspension methods produced mass estimates ten to one hundred times greater than those from the other preparation techniques. The comparison is complicated by the possibility of fiber loss especially for the condensation washer and direct Jaffe wick preparation of grids from Millipore filter pieces without carbon coating.

Table 4. Road Dust Air Samples: Chrysotile Fiber Concentration.

(10^3 fibers/m^3)

Sample	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7
	BDL[a]	BDL	BDL	14	220	3	1350
	BDL	BDL	BDL	BDL	120	20	1530
	BDL	BDL	0.4	26	17	100	1260
	0.8	0.06	2.5	14	270	620	3330
	1.3	2.6	7.3	150	660	1710	2430
	0.2	3.0	4.2	100	340	430	2340
	0.5	4.2		170	2820	1370	5310
	0.2	3.3	16	34	1670	5480	9450
	0.8	6.9		27	920	3070	10200

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[a] BDL = Below detection limit.

Grid preparation technique used by each laboratory:

Lab 1 - Uncoated filter direct transfer by condensation washer.
Lab 2 - Uncoated filter direct transfer by condensation washer.
Lab 3 - Uncoated filter direct transfer by Jaffe.
Lab 4 - LTA/low energy ultrasound/carbon coated Nuclepore/Jaffe.
Lab 5 - LTA/surfactant, ultrasound/carbon coated Nuclepore/Jaffe.
Lab 6 - LTA/high energy ultrasound/carbon coated Nuclepore/Jaffe.
Lab 7 - LTA/rubout.

4. Intralaboratory Comparison of Chrysotile Fiber Concentrations for Different Sample Preparation Techniques

In order to test the EPA provisional method for asbestos in air, the Environmental Monitoring Systems Laboratory at Research Triangle Park, NC recently collected air particulates on 0.4 μm pore size Nuclepore filters in the vicinity of asbestos mining operations [17]. Eight by ten inch Hi-Vol, 37 mm personal, and 102 mm membrane samplers were used. The filters were carbon coated prior to shipment. Direct preparation from these filters with the Jaffe wick technique produced grids which when examined by TEM revealed very light and non-uniform particle distribution. A set of samples containing the most chrysotile was chosen for detailed study. These 37 mm filters had been collected simultaneously on side-by-side personal samplers with filtration times regulated to produce samples with 0.0, 0.017, 0.034, and 0.60 cubic meters of air per square centimeter of filter. Thus, the greatest volume of air per unit area of filter was twenty times less than for the air samples reported in Table 4.

TEM grids prepared by Jaffe wick direct transfer of carbon coated Nuclepore filter pieces were difficult to examine for chrysotile fibers because of non-uniform distribution of particles and clusters of fibers and fibrils which were imperfectly sized and counted with procedures provided by the provisional method. Some of the complex chrysotile fiber groupings on the filter were suggestive of fiber separation upon impact of loose clumps of short fibrils on the filter surface (figure 3a). Other chrysotile fibrils appeared to be either attached to the surface of other mineral particles or locked within a non-chrysotile mineral matrix (figure 3b).

In order to determine if LTA and resuspension of the samples could improve the fiber distribution for counting and evaluate the influence of gentle resuspension techniques on fiber concentration estimates, two pieces of each Nuclepore filter were submitted to LTA. One subsample ash was resuspended in filtered water by five minutes of ultrasonic bath treatment and the other subsample ash was resuspended in filtered water by five minutes of swirling with a vortex device. Ashed air sample suspensions were filtered on to 25 mm 0.1 µm pore size Nuclepore filters, rather than 47 mm filters, in order to increase the particle loading on the TEM grids by a factor of almost four. This nevertheless produced a particle loading approximately one sixth that on the original filters. A comparison of chrysotile fiber concentrations obtained for the three different sample preparation techniques is provided in Table 5.

The previously mentioned problems with sizing and counting fibers and clumps, non-uniform distribution of particles on the original filters, and the small number of fibers counted for some samples contributed to poor precision. Operator difficulty with existing guidelines for counting chrysotile fiber clumps and loosely associated fibril aggregates contributed to some of the largest differences. For example, there were more single fibrils counted for sample 2312/direct preparation as evidenced by the very small mean and median fiber size. Thus that sample has a large fiber number concentration but a very small mass concentration as a result of data based on an estimate of the number of fibrils in each of several aggregates observed. In other samples fibrils tended to occur more in bundles or clumps that were counted as single large particles. Chrysotile identification was based primarily on morphology rather than positive selected area electron diffraction of each fibril. Median fiber widths were slightly reduced for both resuspension techniques as compared to the direct preparation.

The LTA/resuspension techniques may have separated loosely associated fibrils to an extent that some were counted as separate particles rather than parts of clumps. An overall two- to three-fold increase in chrysotile fiber number concentrations for the resuspension techniques was observed in contrast to differences of several orders of magnitude reported for some other preparation procedures in Table 4. Chrysotile mass concentrations from individual sample analysis are quite divergent from mean values for the three analyses of each sample. This is not surprising in view of the statistical problem of estimating mass concentrations from samples containing large, complex chrysotile fiber aggregates which are few in number but represent a very large fraction of the chrysotile mass. The mass concentrations for each sample preparation technique as calculated for the sum of the four air samples were in better agreement perhaps because of the larger number of particles contributing to each estimate.

Sample 2310 was a blank filter sample. The LTA/sonify subsample contained 20 chrysotile fibrils in 10 grid squares examined. The blank chrysotile fiber concentration calculated from the average air volume for the four LTA/sonify preparations equals one fourth the mean of the four sample estimates. Thus contamination, probably from the cross sample contamination during LTA or from ashed filter residues [11], may significantly raise the fiber concentration particularly when few fibers are counted per grid square.

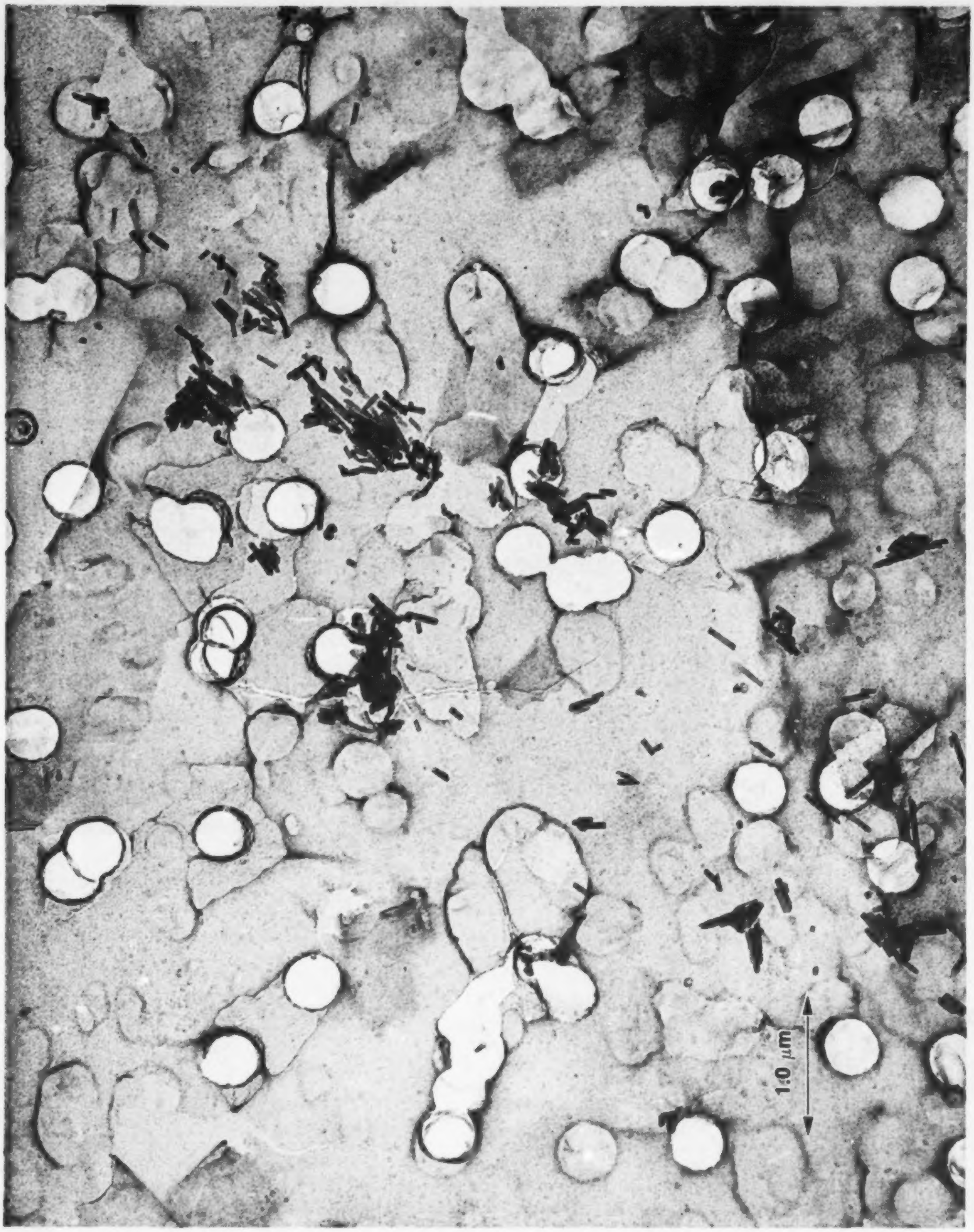

Figure 3a. Electron micrographs of air sample preparations (carbon-coated Nuclepore filter direct transfer) presenting fiber counting problems. Non-uniform distribution of very small chrysotile fibrils and clumps of fibrils.

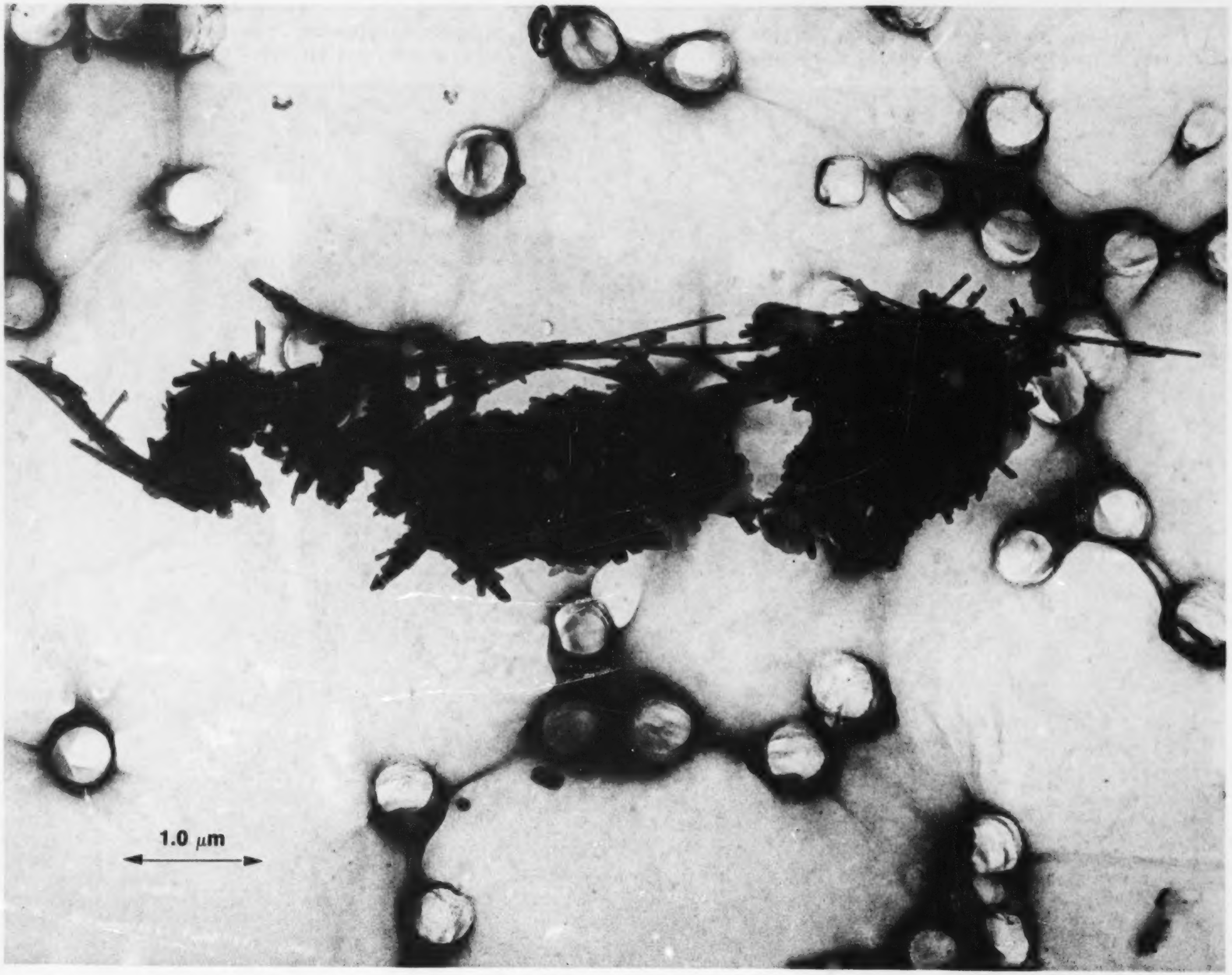

Figure 3b. Electron micrographs of air sample preparations (carbon-coated Nuclepore filter direct transfer) presenting fiber counting problems. Complex clumps of chrysotile fibrils with non-chrysotile mineral matrix.

Table 5. Chrysotile Fiber Concentrations for Air Samples Prepared Both by Direct Transfer from Nuclepore Filters and LTA/Gentle Resuspension Techniques.

Sample	Technique	Filter Area Ashed (mm^2)	Air Volume (m^3/cm^2 Final Filter)	Grid Squares counted	Chrysotile # Fibers	Chrysotile 10^6 Fibers/m^3	Chrysotile $\mu g/m^3$	Mean Length (μm)	Mean Width (μm)	Median Length (μm)	Median Width (μm)
2310	direct		0	10	3	0.09	0.0003	0.43	0.05		
	sonify	39.73	0	10	20	3.08	0.0138	0.31	0.05	0.30	0.05
	vortex	66.86	0	10	0	<0.17					
2322	direct		0.01719	10	37	2.27	1.49	1.23	0.16	0.70	0.10
	sonify	51.53	0.00354	10	44	13.1	0.265	0.97	0.08	0.60	0.06
	vortex	29.24	0.00201	15	19	6.63	8.19	1.30	0.20	1.00	0.06
2312	direct		0.02725	10	222	8.58	0.084	0.40	0.04	0.20	0.03
	sonify	49.64	0.00541	10	46	8.95	0.0980	0.67	0.07	0.60	0.06
	vortex	41.24	0.00450	10	36	8.42	2.21	1.17	0.16	0.90	0.08
2315	direct		0.03450	10	57	1.74	1.13	1.25	0.19	1.00	0.08
	sonify	38.37	0.00530	10	111	22.1	2.87	1.04	0.11	0.70	0.07
	vortex	33.76	0.00466	10	113	25.5	1.82	0.61	0.09	0.45	0.05
2325	direct		0.05977	10	190	3.35	3.95	0.74	0.14	0.80	0.08
	sonify	54.52	0.01303	10	111	8.97	7.58	0.86	0.11	0.60	0.06
	vortex	54.19	0.01296	10	70	5.69	1.01	0.91	0.09	0.45	0.06
Sum	direct		0.12702	40	506	4.19	2.39	0.68	0.10	0.72	0.10
	sonify	233.79	0.02728	40	312	12.0	4.23	0.91	0.10	0.61	0.07
	vortex	225.29	0.02412	45	238	9.23	2.07	0.85	0.11	0.50	0.06

5. Conclusions

Interlaboratory comparisons of mineral fiber concentrations determined by electron microscopy for air particulates collected on membrane filters have been shown to be greatly influenced by sample preparation procedures. Large systematic and predictable differences exist between results obtained by direct transfer of particles from the filter to TEM grids and results from grids prepared following harsh resuspension procedures which cause fiber size reduction and thus greatly increased numbers of particles. Fiber loss during direct transfer preparation of TEM grids is prevented by use of the carbon coated Nuclepore filter/Jaffe wick technique. Resuspension procedures following low temperature ashing of membrane filters can be chosen to minimize but not prevent chrysotile fiber comminution while removing interfering material, optimizing particle loading and uniformity of distribution, and allowing air particulate collection on ashable membrane filters other than the Nuclepore type.

Improvement in methodology for chrysotile fiber analysis of air samples requires work in a number of areas. The development of optimum sampling strategies and techniques is needed. The definition of what sizes of particles and clumps are hazardous and the need for fiber mass measurements must be made. This information will assist in the development of better guidelines for counting and sizing fiber bundles, clumps, and aggregates. Finally, suitable standard samples are necessary for interlaboratory tests of proposed methods.

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DEVELOPMENT OF AN ASBESTOS REFERENCE SUSPENSION

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Abstract

The objectives were: consistent asbestos fiber numbers and sizes per vial, fiber sizes similar to those in field samples, fiber loading easy to count, no clumps of fibers, no debris, no significant physical and chemical deterioration. Methods included separation and suspension of fibers in 0.1 percent Aerosol OT surfactant, fiber length selection/production by high-power ultrasonication, and uniform dispersal of fibers in surfactant by reciprocal shaking. Vials were used instead of ampoules for quantitative transfer of the 25 mL contents. A filtration "buffer" promoted even dispersal of fibers on filters. Filters were not dried, in order to prevent fiber losses before carbon coating. Low-power ultrasonic baths may cause separation of fibrils. The objectives were met.

1. Introduction

There has been growing interest in and concern about the occurrence of asbestos in drinking water during the past several years. This has resulted in: 1) an increasing number of laboratories analyzing water for asbestos, and 2) continuing evolution of the analytical technique. As must be expected with such a relatively new technique that continues to be adopted by more laboratories and undergo modifications in various laboratories, uniform application of the technique does not exist between laboratories.

An asbestos reference suspension would provide a tool for determining the adequacy of the presently accepted analytical techniques and for improving the techniques. A reference sample would also provide a means to evaluate and compare laboratory capability.

The research presented here to develop an asbestos reference suspension, had several objectives in the following general order of priority:

- consistent numbers of asbestos fibers per vial or ampoule
- consistent fiber size distribution per vial or ampoule
- fiber sizes similar to those found in field samples
- fiber loading easy to count and analyze
- no clumps of fibers
- no debris
- no significant physical and chemical deterioration.

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1.1 Initial Approach

Initially, the goal was to accomplish all of the objectives simultaneously. Because most of the objectives, except for chemical stability of the fibers seemed to have been achieved or could easily be achieved (based upon work by previous researchers in this laboratory), the primary emphasis was placed on finding the optimum pH to achieve stability [1][2].

The original procedure utilized 2 mg NIEHS #007C chrysotile that was stirred in 0.5 percent Aerosol OT® [3] for 16 hours to separate the fibers. This suspension was ultrasonicated at 200-300 watts with a Biosonik IV (VWR Scientific) for 30 minutes to break the fibers. A portion of the sonicated suspension was diluted and its pH adjusted; a subset then buffered at 10.0, 10.5, or 11.0. While the suspension was being stirred, 20 mL aliquots were withdrawn and placed in ampoules. Two sets of ampoules calculated to contain 36 or 40 ng of chrysotile were prepared. Mercuric chloride was present throughout the procedure as 27.1 mg/L for bacteria control.

The results from using this procedure consisted of: (1) reduced fiber numbers and increased fiber length when sonication power was reduced from 300 to 200 watts; (2) the percent diffraction of the fibers being higher at pH 11 than at 10.5; (3) a wide variance in fiber concentration among the ampoules; and (4) the suspensions containing clumped and distorted fibers, and debris.

The most critical problems that arose were uneven distribution of fiber numbers and sizes, and the occurrence of clumps. These problems eliminated the quantitative use of the reference, and were much more significant problems than reduction of percent diffraction. (This reduction could have been caused by the low pH Aerosol OT® leaching magnesium from chrysotile. Analysis of the filtered solution of one ampoule, five months after preparation, gave a concentration of dissolved Mg that approximated 5.8 percent of the Mg that should have been in the chrysotile.) The pH adjuster and buffer solution (NaOH and $NaHCO_3$) may have been responsible for some of the problems observed since they change the charge on chrysotile (the isoelectric point of chrysotile is pH 11.8).

Corrective approaches included the lowering of the pH to 9 and 10, using Teflon® vials (to avoid possible dissolution of glass), finding an effective alkaline surfactant, and reducing sonication power and time. Some of these approaches were applied simultaneously. The Teflon® vials produced a new type of smooth, fibrous debris. The alkaline surfactants tried were either incompatible with polycarbonate filters (sodium oleate) or ineffective in separating and evenly suspending chrysotile (Triton RW-100®). Sonication was still too powerful at 150 watts, since too many fibers were produced and too many were small fibers. Debris and distorted fibers were also present. Additionally, deterioration of the sonic probe released titanium particles into the suspension.

1.2 Redirection

Because of the inability to attain the project objectives with the above approaches, plus the need to use a different model sonicator that had a probe in good condition, major changes were made in the procedure. All aspects of the original procedure were evaluated through experimentation, and eventually resulted in a new procedure that produced a satisfactory asbestos reference suspension.

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[2] Figures in brackets indicate the literature references at the end of this paper.

[3] Mention of trade names of commercial products does not imply endorsement by the U. S. Environmental Protection Agency.

2. Materials and Methods

2.1 Surfactant

Surfactant is used to separate asbestos fibers and to help keep the fibers suspended. An early reference to the industrial use of surfactants for dispersing asbestos fibers shows that Aerosol OT® is one of the most effective surfactants [2]. Using relatively concentrated suspensions, Novak determined the minimum amount of Aerosol OT® necessary to maintain a stable suspension of a known percentage of chrysotile in the surfactant solution. The plot he presented can be described as:

$$\text{Percent Aerosol OT®} = (0.0376 \times \text{percent chrysotile}) + 0.0343.$$

The reference suspensions contained on the order of 10^{-7} percent chrysotile, so the first quantity can be ignored. For such dilute suspensions of chrysotile, the percent Aerosol OT® needed to maintain a stable suspension is therefore 0.0343 percent.

Novak observed that it was necessary to add more surfactant solution to a chrysotile mixture after several days to maintain fluidity. This indicated that more asbestos surface was being exposed and interacting with the surfactant. With the possibility of this increased surfactant demand with time, plus unknown errors in the determination of the optimum amount of Aerosol OT® for a dilute reference suspension of chrysotile (as opposed to the industrial slurries), 0.1 percent Aerosol OT® solution was used throughout the procedure.

Sodium chloride has been used to make Aerosol OT® more effective in preparing chrysotile suspensions that remained clump-free for at least three months; the solution was 0.1 percent Aerosol OT® plus 0.1 percent NaCl [3]. The manufacturer of Aerosol OT® gives data showing that both interfacial and surface tension of 0.1 percent Aerosol OT® (and other concentrations) are reduced by the addition of NaCl at 0.25 and 0.50 percent with no data at 0.1 percent [4]. With Aerosol OT® of 1.0 percent or greater, these NaCl additions caused clouding, but it was not stated whether the clouding directly affected the ability or indicated a change in the ability of Aerosol OT® to wet and suspend solids.

Chrysotile suspensions prepared with 0.1 percent Aerosol OT® plus 0.1 percent NaCl were not noticeably different than those without NaCl. A solution of 0.1 percent Aerosol OT® plus 0.1 percent NaCl not containing asbestos developed cloudy streaks after a few weeks. With no clear benefit and the possibility of NaCl disrupting the Aerosol OT®, NaCl was not used with this reference.

Distilled, deionized filtered (0.22 µm) water was used for these solutions as well as for washing and rinsing of all equipment, and for all steps in the method that required water.

2.2 Standard Chrysotile

The chrysotile used was acquired from the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. This sample is labeled NIEHS #007C, and is referred to as their short-range chrysotile. The source is Union Carbide Corporation's mine in the New Idria serpentinite mass in California; the company labels the standard chrysotile as COF-25. A detailed description of this material has recently been published [5].

Weighing the standard chrysotile was the most arduous step in the preparation of the reference suspension, particularly because sub-milligram quantities were required. A Mettler M5 Microchemical Balance was used to weigh 0.1 mg chrysotile. Aluminum weighing dishes were used instead of plastic ones to eliminate the problem of static charge buildup. Weighing was done on days with low atmospheric humidity since chrysotile is hydroscopic. To allow for a moisture equilibrium to be reached between the chrysotile and the atmosphere, the vial of chrysotile was opened at least an hour before weighing the 0.1 mg portion. During this period, the stability of the balance was checked.

The weighed chrysotile was added to a screw-capped bottle containing 2 L of 0.1 percent Aerosol OT® and a stirring bar. This stock suspension was stirred for 30 minutes, with a 2-3 cm deep vortex. To dilute this suspension to a desirable concentration, 2 percent of the suspension was withdrawn and diluted. A stirring suspension may have an uneven distribution of fibers, and lead to the withdrawal of unrepresentative aliquots. Small scale tests in this lab with powdered carbon, bottles of various shapes, and shakers (reciprocating and wrist-action), showed that bottles laid length-wise in a reciprocating shaker gave the most random distribution of the visible carbon in water. Thus, the 2 percent withdrawal of suspension was accomplished by shaking the bottle lengthwise in a reciprocating shaker, withdrawing 20 mL, shaking again, and withdrawing another 20 mL. Shaking time was not recorded, but was about one minute each time. The 20 mL aliquots were added to a 350 mL wide-mouth jar containing 200 mL of 0.1 percent Aerosol OT®.

2.3 Sonication

The 240 mL suspension in the wide-mouth jar was sonicated with a Fisher Sonic Dismembrator, Model 300. The intermediate probe was used, and the unit was operated for five minutes at the 60 percent setting, after trying higher and lower settings. Calibration of the sonicator was performed by operating at these same conditions, with the probe immersed in 240 mL 0.1 percent Aerosol OT® without chrysotile. The solution was at equilibrium temperature before sonication, and the temperature was monitored with an electronic thermometer for the five minute sonication. During preparation of the asbestos reference, the jar was placed in an ice bath during sonication, but during calibration the bath was not used.

The output of the sonicator was determined by a relation between mechanical and thermal energy. Assuming 100 percent transfer of the sonic energy to the solution as heat:

$$\text{Increase in thermal energy} = (4.2 \times 10^3 \text{ joules/KgC}^\circ) m \Delta T$$

where 4.2×10^3 joules/KgC° is the specific heat of water, ΔT is the change in temperature of the water, and m is the mass of water [6]. Dividing this energy figure by the period of sonication, in seconds, the power received by the solution is expressed in watts. Assuming the 0.1 percent Aerosol OT® has a specific heat that is nearly that of water [7], this relation yields 15.5 watts or 0.065 watts/mL of 0.1 percent Aerosol OT®. Using 240 mL of distilled deionized filtered water, the values were 16.1 watts or 0.067 watts/mL. Not enough individual calibrations were performed to indicate if the difference between the values for Aerosol OT® and water are significant. During the five minute calibrations, 4.5-4.8 C° temperature changes occurred, with upper limits below 27.3 °C; air temperature averaged 23.0 °C. Under these conditions there was probably negligible heat loss to the surrounding environment [8], so the calibration should be reliable.

2.4 Reference Suspension

The 240 mL sonicated suspension was poured into a glass aspirator bottle. The wide-mouth jar was rinsed with measured volumes of 0.1 percent Aerosol OT® which were added to the aspirator bottle. Then 0.1 percent Aerosol OT® was added to create a 2 L volume of suspension in the aspirator bottle. The bottle had a smooth glass neck that was stoppered with an aluminum foil-covered rubber stopper. The aspiration port was cut and fitted with a screw-top neck using epoxy. The cap for the neck had a Teflon®-coated liner.

The bottle was fastened length-wise into the reciprocating shaker, with the screw-top neck facing upward. This arrangement allowed for the easy withdrawal of aliquots after shaking the suspension. During preliminary tests, 25 mL aliquots were withdrawn with a volumetric pipet and filtered immediately. During preparation of the final reference suspension, the 25 mL aliquots were placed in vials. The suspensions were shaken for about one minute before each aliquot was withdrawn.

The vials, caps, and liners were washed in a sonic bath with Aerosol OT® before using them for the reference suspension. The liners were rubber-backed Teflon® Ampoules had been used for previous references, but they do not allow for the quantitative transfer of the contents. With 25 mL ampoules, the contents must be shaken out (they cannot be poured), some of the fluid is generally trapped in the cap, and rinsing is more difficult than with vials. Ampoules are also often difficult to break open safely. The rubber-backed liners for vial caps create a seal tight enough to replace the melted glass seal of ampoules.

Seventy-two vials were filled and four additional aliquots were taken throughout the vial-filling procedure and filtered directly. The vials were labeled and numbered according to the order of filling. Four vials had their contents filtered the day they were filled, and the rest were boxed and refrigerated. Results from the four vials determined whether the reference suspension was suitable to send to other laboratories.

2.5 Filtration

During preliminary tests, the 25 mL aliquots of reference suspension were filtered directly from the pipet rather than from vials. With the final reference suspension the same was done with four aliquots, plus the filled vial to be filtered was sonicated in an ultrasonic bath (50 watts) for three minutes.

A Schleicher and Schuell glass filtration apparatus, holding 47 mm filters, was used. A cellulosic membrane filter is placed on the glass frit as a backing filter for the polycarbonate filter, which actually collects the fibers. The pore size of the backing filter, is probably not important, though 0.45 μm is the smallest that would normally be used, just for the convenience of not slowing the filtration. (Further study may reveal that there is an optimum filtration rate to achieve the most even distribution of particles on the filter.) Another researcher at the Environmental Research Center [9] discovered that an effective way of placing the Nuclepore polycarbonate filter (0.1 μm) to avoid wrinkling and folding, is to cover the backing filter on the frit with a bubble of water, lay the Nuclepore filter on the bubble, and apply vacuum. The glass funnel is attached firmly, and then the vacuum valve closed (vacuum is still maintained in the filtration flask for some time).

Prior to adding the reference sample to the funnel, approximately 25 mL of water is added to the funnel; the reference sample is then added and the vacuum is applied. This technique was found to give more even distribution of fibers on filters than filtering the reference without first adding the 25 mL "buffer". The water buffer was also slightly more effective than a 0.1 percent Aerosol OT® buffer (25 mL). (See section 3.1.) Although this is somewhat surprising it is more convenient for laboratories to add only a water buffer, and not Aerosol OT®.

The vials are rinsed three times with water, and the rinses are added to the filtering reference. The funnel is covered with aluminum foil during filtration.

2.6 Filter and Grid Preparation

Once the reference sample has been filtered completely, the vacuum is closed, and the filter is gently lifted to release the remaining vacuum pressure. The filter is not dried, but is enclosed in a 50 mm diameter tight-fitting petri dish; a dried filter may lose fibers easier. Petrographic glass slides are conveniently sized for attaching a quadrant of the filter and a label. The edges of the quadrant are attached to the slide with tape or drops of chloroform. Care is taken to handle the filter gently and to keep it horizontal to avoid loss of fibers. Two or three slides are placed in a petri dish for transport and for later storage.

A Denton vacuum evaporator is used in this laboratory to carbon coat the filter quadrants. The evaporator is operated at a constant current of 30 amp and a potential of 60 V for no more than 10 seconds. The slides are not rotated during operation of the evaporator.

The arrangement used to prepare grids is the necessary number of specimen grids (200 mesh) lined up on a stainless steel screen (200 mesh, approximately 3 x 3 cm) that is laid on a cleaned glass slide. A section of carbon-coated filter measuring approximately 2 x 2 mm is cut with a cleaned razor blade, grasped on an edge with self-closing tweezers, passed before a static charge eliminator, and then laid squarely on a grid with the coated side of the section facing against the grid. A drop of chloroform is released onto the section and grid with a microliter syringe to seal the section to the grid. This is repeated with all the coated filters, usually preparing two grids per filter quadrant. The screen, with the grids upon it, is then transferred to the chloroform wick.

The chloroform wick used is a modification of the Jaffe wick [10], replacing the glass slides and filter papers with a polyurethane sponge. The screen supporting the grids is placed on the sponge. Roughly 24 hours are needed to dissolve the filter material away from the sections.

2.7 TEM Analysis

The initial analyses of the preliminary reference samples and of the first four reference vials were conducted to determine whether the primary objectives had been met. Therefore, the chrysotile was identified only by morphology, not by crystal structure or elemental composition. The data from these initial analyses also enabled determination of a statistically suitable number of fibers or grid squares to count.

When analyzing a reference, three distantly spaced grid squares are observed on each of two grids. A diffraction pattern is attempted on each fiber found. The fiber is sized and listed as either diffracting chrysotile, non-diffracting chrysotile, or something else, e.g., amphibole. If the width of the fibers are 0.1 µm or greater they are noted with the lengths of the corresponding fibers. Irregular chrysotile and debris should be noted. Each grid square observed is measured to give an accurate determination of fiber concentration. This measurement is done at low magnification on the TEM. Observation of fibers is best performed between 14,000 and 20,000 X.

3. Results

3.1 Preliminary Test

The preliminary test began with 0.077 mg of chrysotile, which was a quick random measurement close enough to 0.100 mg. (With the standard chrysotile formed into various-sized small balls, it is difficult to weigh out the exact amount needed in such small quantities.) No vials were used; the aliquots were filtered from the pipet. Three series of filtrations were carried out, each with three aliquots, i.e., three filters: 1) 25 mL water buffer; 2) 25 mL 0.1 percent Aerosol OT® buffer, and 3) no buffer. Two grids were prepared and analyzed from each filter.

The data show that filtrations with the water buffer produced the most consistent results (Table 1).

Table 1. Variation in Chrysotile Count with Three Filtration Techniques for an Asbestos Suspension.

	25 mL Water Buffer	25 mL 0.1 percent Aerosol OT® Buffer	No Buffer
No. grid squares	23	20	25
Fibers/grid square, $\bar{x}$	8.91	8.45	8.44
Standard deviation	2.83	3.44	4.27
Range	3-16	3-16	2-17

The fiber numbers per grid square were satisfactory and could be twice as numerous without creating difficulties in counting and analyzing. The fiber lengths ranged from 0.2 µm to about 12.0 µm, and there were adequate numbers of bundles of 0.1 µm and wider; clumps were absent. The overall appearance of the grids was good. These results encouraged the preparation of the reference suspension to be distributed to other laboratories.

3.2 Final Suspension

The final suspension was prepared with 0.100 mg chrysotile yielding 25 ng per vial or per 25 mL aliquot. During the filling of 72 vials, four additional 25 mL aliquots were filtered directly from the pipet. Four vials (numbers 5, 23, 39, and 56) were also selected from the 72 vials and filtered a few days later. Vial number 5 did not produce satisfactory grid preparation; all the squares were empty, possibly because of a defective filter (the filters and grids prepared for the four vials were prepared under identical conditions). The results for the three vials and the direct filtrations are in Table 2.

Table 2. Chrysotile Count in the Final Asbestos Reference Suspension.

	Direct Filtration	From Vials
Number of filtrations	4	3
Total number of grid squares	28	24
Fibers/grid square, $\bar{x}$	9.64	19.25
Standard deviation	3.14	6.97
Range	1-16	12-34

Within the two categories the results were consistent. The overall appearances were good; only a few clumps occurred, but these had countable numbers of fibers. The vials yielded twice as many fibers as the direct filtrations. More detailed data is available for the preliminary test and the final suspension.

4. Discussion

Both the direct filtrations and the filtrations from vials exhibited the desired major objectives. Debris was minimal, and physical and chemical deterioration of the fibers was not evident. Only after several months of shelf-life can a real evaluation be made, however.

Realizing that different results may arise between direct and vial filtrations, direct filtrations were repeated when the final reference sample was prepared to confirm the results from the preliminary test. The results were similar with mean counts of 8.9 fibers/grid square for the preliminary test (water buffer) and 9.6 fibers/grid square for the reference. The difference can be partially attributed to the greater amount of chrysotile used for the final reference (0.077 mg vs. 0.100 mg).

The discrepancy between the direct filtrations and the vial filtrations may be due to four factors: 1) unrepresentative sampling - this is unlikely because both the direct filtration and vial filtration aliquots were taken at intervals throughout the filling of 72 vials; 2) contamination of vials - the vials, caps, and liners were scrupulously washed with filtered Aerosol OT®, distilled deionized, filtered water, and an ultrasonic bath and protected, however; 3) time - the vial references were filtered two and a half days after the direct filtrations; the extra time may have allowed for more separation of fibers in the vials; 4) ultrasonic bath - the three minute sonication at 50 watts of the vials should not be enough to break fibers, but may have caused separation of fibers.

During the development of this asbestos reference suspension, discrepancies in asbestos counts between samples from the same suspension were always accompanied by differences in fiber size distributions between the samples. This was evident between the direct filtrations and the vial filtrations for this reference, too (Figure 1). The direct filtrations had more fibers in the longer classes than did the vial filtrations. The mean lengths were 2.6 and 1.8 µm, respectively. The direct filtrations had 23 percent of the fibers with widths of 0.1 µm or greater, whereas the vial filtrations had a corresponding figure of 20 percent.

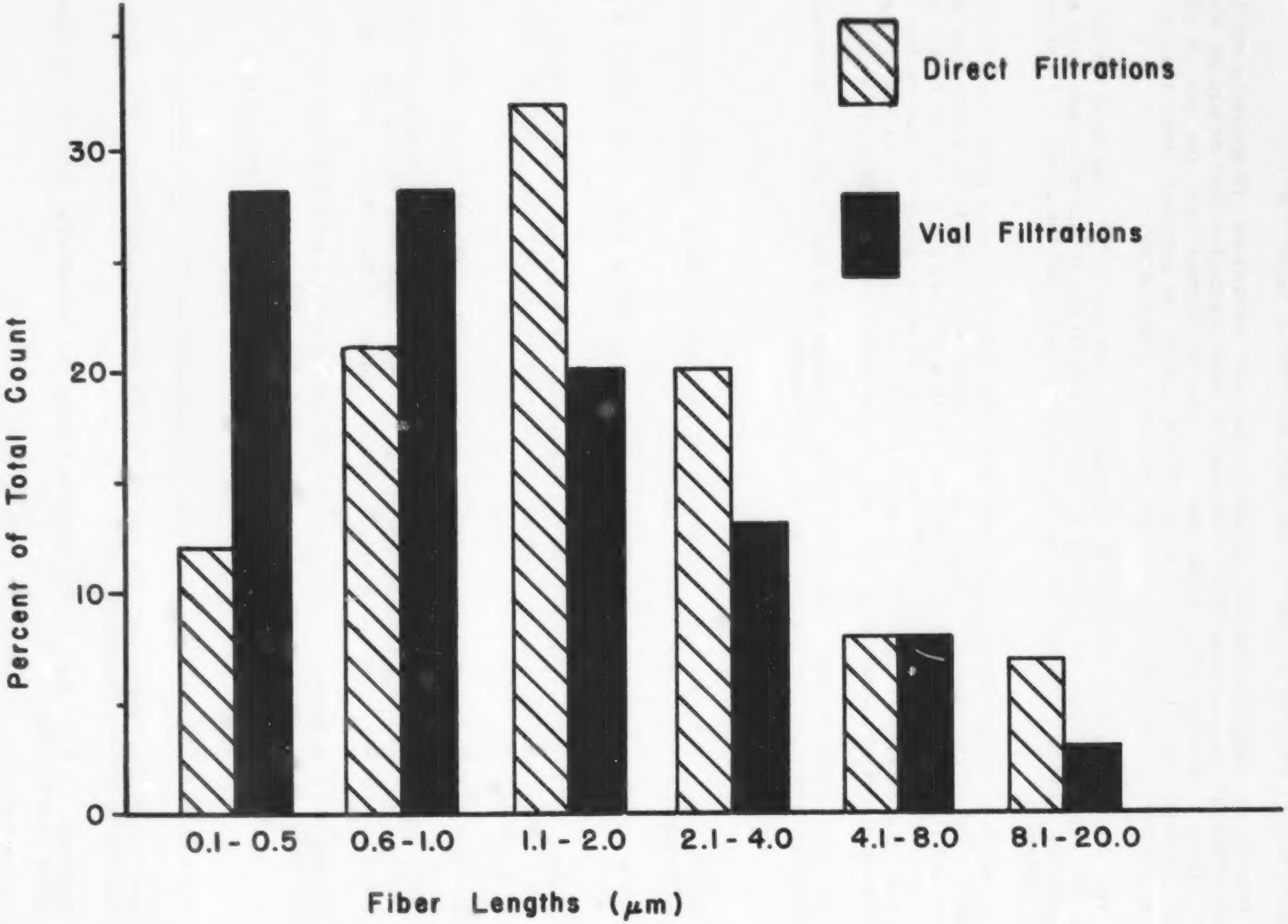

Figure 1. Fiber length distribution of asbestos reference suspension.

The most reasonable explanations for the size discrepancy is that during the low-level sonication of the vials, fibers separated, creating the lower percentage of wide fibers. Because many fibers include fibrils of different lengths, the separation of fibers probably released many of the smaller fibrils that were attached to longer fibrils.

Despite the apparent changes caused by low-power sonication, it probably has the beneficial effect of resuspending fibers that may be adhering to the vial or may be adhering to other fibers. Unfortunately, there was not time to further test the use of ultrasonic baths. However, any effect of ultrasonic baths should be constant, from vial to vial, as long as the sonication conditions are the same, e.g., power output.

The vials were sent to over 20 laboratories for analysis. The decision to send the vials was based upon the consistency of results within the groups of suspensions analyzed, and upon the agreement between the preliminary suspension and the direct filtrations of the final suspension.

A complete set of instructions was sent with each vial as a guide for filtration and analysis. Data from vial filtrations in this study were used to determine how many grid squares should be observed by each analyst. With approximately 2.7×10^6 fibers per vial[4] and a pooling of the variance for each vial, the analysis of six grid squares for each vial resulted in a confidence interval of approximately 0.6×10^6 fibers per vial. Analysis of six squares is not an overwhelming task and the confidence interval was acceptable.

The next stage of this study will be to evaluate the data from the laboratories that analyzed the reference. Initial returns are encouraging.

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[4] $$\frac{\text{Filterable area of filter}}{\Sigma(\text{grid square areas observed})} \times \text{number of fibers counted}$$

$$\frac{1075 \times 10^6\ \mu m^2}{1944 \times 10^4\ \mu m^2} \times 482 \text{ fibers} = 2.7 \times 10^6 \text{ fibers/vial}$$

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PREPARATION OF AIRBORNE ASBESTOS STANDARDS

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Abstract

The methods used to generate a set of standard hi-vol and personal samples for use in comparison of analytical methods is described. Various problems of predicting the filter loading, minimizing external contamination, and generation of aerosolized fibers are discussed.

1. The Preparation of Laboratory Filters of Controlled Asbestos Loading

1.1 Introduction

A statistically designed study to evaluate the electron microscope analytical methodology for determining asbestos required that filters be prepared under controlled conditions to obtain three asbestos concentrations. Both polycarbonate (Nuclepore) and cellulose ester (Millipore) filters, with pore diameters of 0.2, 0.4, and 0.8 μm, were used and samples were collected using high-volume samplers (with 20 cm x 25 cm filters) and personal samplers (with 3.7 cm diameter filters).

The filters could be prepared in several ways, although it is preferable to obtain simultaneous sampling for different filter types, pore size, and sampler. The methods available to prepare filters are:

- taking samples close to a natural source, varying flow rate and sampling time;
- preparing stock suspensions of known asbestos fiber concentration by ultrasonic treatment of asbestos in water and filtering from the liquid suspension(s);
- aerosolyzing asbestos fibers and sampling from the aerosol cloud.

Sampling from a natural asbestos source (for example, an asbestos products factory), would be the most convenient but, unfortunately, it has the serious disadvantage that the concentration of the source is not known and significant quantities of extraneous particles are also present.

Filter samples can be prepared from liquid suspension(s) of known concentration of asbestos minerals. The disadvantage of this method is that the deposition of the fibers from water suspension onto a filter may not be equivalent to that obtained from an aerosol cloud.

Simultaneous sampling from an aerosol cloud of known concentration is the best technique since it simulates normal sampling conditions while allowing control of the aerosol concentration and filter loading.

1.2 Experimental

The Aerosol Chamber--

The aerosol cloud was generated into a spherical chamber fabricated from welded steel plate with a diameter of 5.5 m and a volume of 86 m^3. The inside of the chamber is coated with an epoxy-phenolic material (Plasite 7122) to prevent corrosion and to provide a smoother finish. The chamber can be cleaned by a hot water spray to wash down the walls, and a high volume extraction system purges the chamber air through an absolute filter device at the rate of 12 air changes per hour.

Figure 1 shows the internals. Three high-volume samplers and six personal samplers were mounted on a catwalk located midway on the chamber walls. The aerosol cloud entered the chamber from the generator located outside the chamber. A fan inside the chamber circulated air to ensure a uniformly mixed aerosol.

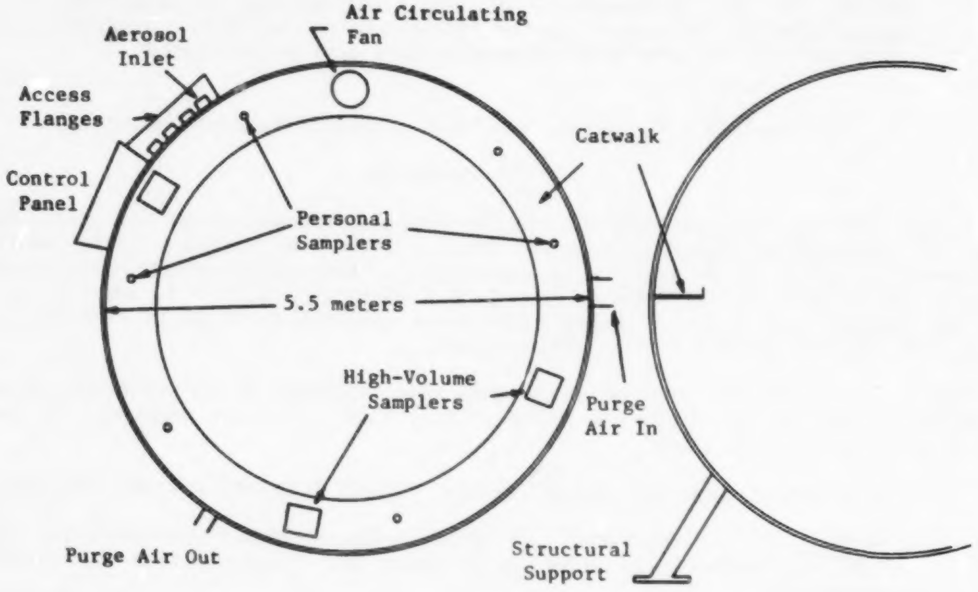

Figure 1. Top view and side view of aerosol chamber showing location of apparatus.

Ultrasonic Treatment to Break Fibers to a Sufficiently Fine Size--

The UICC asbestos minerals have a very coarse particle size, which is unsuitable for charging in an aerosol cloud. Three ultrasonic devices were tested to determine their efficiency in breaking up asbestos into fibers under 10 μm in length. They were:

- Ultra-Sonic Industries - System Forty
  80 Watts Bath Type;
- Polytron Cell Disruptor - PT10
  5000 Watts with High Speed Agitator;
- Branson Sonifier - W 185C
  100 Watts Horn Type.

Tests were conducted by weighing out a small quantity of asbestos and suspending it in distilled water to give an asbestos concentration of about 0.3 percent by weight. Aerosol OT was added as a dispersing aid at a concentration of about 0.2 percent by weight. Ultrasonics were applied for time periods of 5, 10, 20, and 30 minutes using each device. Each sample was then diluted to a concentration of 0.03 percent with filtered distilled water.

The Branson Sonifier was the only unit found suitable for achieving small enough fiber lengths in chrysotile asbestos. By varying the time of the ultrasonic treatment, the chrysotile asbestos could be reduced to any fiber length desired. The most satisfactory chrysotile dispersion was produced by a 45 minute treatment at 100 watts power to 250 mg of asbestos suspended in 150 mL of water with 2 percent of Aerosol OT added as a dispersing agent. The quality of the treatments were checked by both optical and electron microscopes.

The Branson unit was found to be less effective with amosite asbestos and fiber glass. A series of hand-grinding experiments were performed using an agate pestle and mortar. A technique was developed which led to satisfactory dispersion of both amosite and fiber glass. It consisted of wet hand-grinding a 100 mg of quantity of fiber in a few drops of 1:1 solution of water and Aerosol OT for 30 minutes.

Aerosol Generation--

The Sierra Instrument Company's Model 133G Fluid Atomization Aerosol Generator utilizes air-blast atomization and inertial impaction to produce aerosols. It could produce droplets at rates up to 10^9 particles per second. The droplet size was variable from 0.03 to 3 μm diameter.

The generator is schematically illustrated in figure 2. It consisted of a dryer, a pressure regulator, an absolute filter, an adjustable valve, two precision flowmeters, a fluid atomizer, an impactor, and an ionizer.

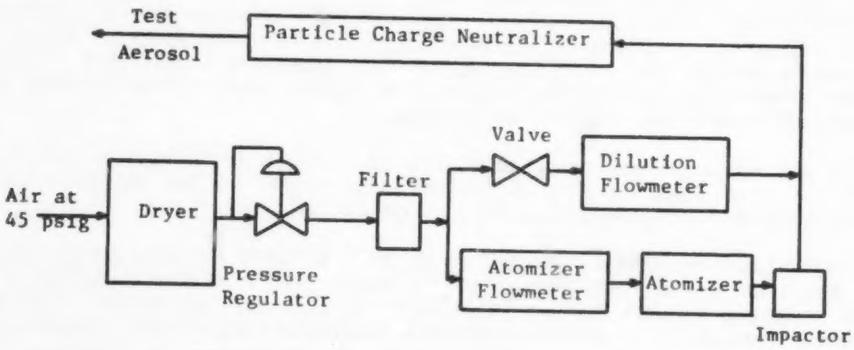

Figure 2. Flow diagram of aerosol monitor.

High pressure air is supplied to the generator at a minimum pressure of 45 psig. The air passes through a chemical dryer and a pressure regulator which reduces the pressure to 35 psig. The air then flowed through an absolute filter and was subsequently divided into two fractions: the atomizer air and the dilution air.

The atomizer air flows through a flowmeter and a Collison-type atomizer. As the air passed through the nozzles of the atomizer, it produces a spray of the suspension directed against a baffle. The spray is then carried by the air through an impactor where large droplets are removed, leaving an aerosol of a narrow size distribution. The remaining droplets then flow to a mixing tee located upstream of the ionizer.

After flowing through the filter, the dilution air flows through a manually adjusted valve. It then passes through a flowmeter and into the mixing tee. From the mixing tee, the solvent is evaporated and the diluted aerosol flows into the ionizer where it is mixed with bipolar ions. The aerosol then exhausts through the outlet located on the side of the generator housing. Care was taken to adjust the fiber liquid concentration to a point where each droplet formed would contain 0 or 1 fiber the vast majority of the time. This precaution is required to minimize agglomeration or clumping of the fibers in the final aerosol. The ionizer employs a radioactive source (1 milli-curie of Krypton 85 gas) to neutralize any static charge developed on the particles in the generator.

During preliminary runs, contamination of the aerosol by the high-volume samplers was observed. The brushes of a hi-vol sampler wear rapidly and, as they wear, emit large numbers of very small carbon particles. The carbon particles deposit on the sample collection media and interfere with the analysis of the filters. There are two ways to solve this problem:

- separate the hi-vol collectors from the motors and bring the motors outside the chamber;
- adjust the chamber concentration to bring the total operating time of the hi-vols to an acceptable level of brush wear particles.

For the particular set of samples, time and money dictated that we use the second remedy.

The requirement that high-volume sampling time be kept below a total of one hour, coupled with the failure of aerosol generators producing more droplets to provide an adequately dispersed aerosol, required a modification of the aerosol generator. Provision was made to pump asbestos slurry, whose concentration was adjusted to compensate for the fiber loss and evaporative water loss, into the atomizer unit. The Sierra Aomizer was thus made operable for periods of 16 to 80 hours on a continuous basis using this make-up system.

This method is useful when the fiber sizes are small enough to remain suspended nearly indefinitely with adequate air circulation in the chamber. It produced excellent relative loadings of single fibers when used with chrysotile asbestos fibers in the size range found in ambient air. When working with the amphibole asbestiform minerals or fiberglass, the comminution procedures result in a higher level of non-fibrous particles and the sedimentation rates are significantly higher.

There are two problems associated with the higher sedimentation rates. The higher sedimentation rate gives rise to a greater uncertainty in the aerosol concentration of the fibers and some fibers may settle onto filter collection surfaces.

We feel that independent generation of the amphibole asbestos aerosol could minimize some of the difficulties experienced or a cleaner source of small amphibole fibers could be used. However, efforts to use other aerosol atomizers of higher capacity to obtain higher concentrations in a shorter time were ineffective since poor dispersion of individual fibers resulted. Such air samples would be unsuitable as standards for electron microscopy work.

1.3 Details of Experimental Work in Samples Prepared in Chamber

In all, 27 samples were prepared as detailed in Table 1. Each sample was unique and a filter was individually prepared.

Table 1. Phase 1 Experimental Plan.

	Independent Variables										
	X1	X2	X3	X3	X5	X6	X7	X8	X9	X10	X11	X12
Sample	Compo.	Loading	Sampler	NP/MP	Pore Size	Particle Side Down/Up	3 mm Location	C-Coat	Filter Removal	Magnifi. 1,000X	Grid Opening Loc.	Choice of Field
1	1	Low	P	M	0.22	--	Ctr	--	Sox 1	10	MR	Random
2	1	Low	P	M	0.45	--	Peri	--	Sox 2	5	Peri	Full Grid
3	1	Low	P	M	0.8	Up	MR	Yes	J	20	Ctr	Consecutive
4	1	Med	HV	N	0.2	--	Peri	--	J	10	Ctr	Consecutive
5	1	Med	HV	N	0.4	--	MR	Yes	Sox 1	5	MR	Random
6	1	Med	HV	N	0.8	UP	Ctr	--	Sox 2	20	Peri	Full Grid
7	1	High	P	M	0.22	--	MR	Yes	Sox 2	10	Peri	Full Grid
8	1	High	P	M	0.45	--	Ctr	--	J	5	Ctr	Consecutive
9	1	High	P	M	0.8	Up	Peri	--	Sox 1	20	MR	Random
10	2	Low	HV	M	0.22	--	Ctr	--	Sox 2	20	MR	Consecutive
11	2	Low	HV	M	0.45	Up	Peri	Yes	J	10	Peri	Random
12	2	Low	HV	M	0.8	--	MR	--	Sox 1	5	CTR	Full Grid
13	2	Med	P	M	0.22	--	Peri	Yes	Sox 1	20	Ctr	Full Grid
14	2	Med	P	M	0.45	Up	MR	--	Sox 2	10	MR	Consecutive
15	2	Med	P	M	0.8	--	Ctr	--	J	5	Peri	Random
16	2	High	P	N	0.2	--	MR	--	J	20	Peri	Random
17	2	High	P	N	0.4	Up	Ctr	--	Sox 1	10	Ctr	Full Grid
18	2	High	P	N	0.8	--	Peri	Yes	Sox 2	5	MR	Consecutive
19	3	Low	P	N	0.2	Up	Ctr	Yes	J	5	MR	Full Grid
20	3	Low	P	N	0.4	--	Peri	--	Sox 1	20	Peri	Consecutive
21	3	Low	P	N	0.8	--	MR	--	Sox 2	10	Ctr	Random
22	3	Med	P	M	0.22	Up	Peri	--	Sox 2	5	Ctr	Random
23	3	Med	P	M	0.45	--	MR	--	J	20	MR	Full Grid
24	3	Med	P	M	0.8	--	Ctr	Yes	Sox 1	10	Peri	Consecutive
25	3	High	HV	M	0.22	Up	MR	--	Sox 1	5	Peri	Consecutive
26	3	High	HV	M	0.45	--	Ctr	Yes	Sox 2	20	Ctr	Random
27	3	High	HV	M	0.8	--	Peri	--	J	10	MR	Full Grid

Footnotes:
P = Personal
HV = High-volume
Ctr = Center
Peri = Periphery
MR = Mid-radius
Sox 1 = Short
Sox 2 = Long
J = Jaffe
C-Coat = Carbon Coat
M = Millipore/Cellulose ester
N = Nuclepore/Polycarbonate

The filters were prepared in three chamber runs, collecting nine filters per run. A detailed schedule for each run was prepared in advance using standard engineering techniques. While it is fairly reasonable to assume that removing 2 L/min with several personal samplers will not produce a significant change in concentration in an 86,000 liter aerosol chamber, it is also reasonable to assume that 60 min. of hi-vol operation at about 500 L/min, a total of 30,000 L will result in a drop in concentration.

During the study, we first assumed, then demonstrated, that a good estimate of the concentration at any given time could be made by treating the chamber as a well-mixed batch reactor. If the chamber is a well-mixed batch reactor, the hi-vol streams become a dilution stream entering the reactor. The mathematical expression describing the concentration in the situation is an exponential decay equation whose constant is proportional to the total instantaneous flow of air through the hi-vol samplers. The flow rates through the hi-vol samplers were dependent on both filter type and pore size, as seen in the following measured flow rates, as shown in Table 2.

Table 2. Flow Rates of High-Volume Samplers[a]

	Flow Rate (L/min)	
Nominal Pore Size	Polycarbonate	Cellulose Acetate
0.2	651	396
0.4	708	453
0.8	764	679

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[a] These results are independent of the sampler actually used, and remained constant for every sampling run.

Combining the concentration-time equation with two other criteria permitted us to prepare the series of 27 filters in three runs. The two criteria which defined the experimental conditions were:

1. Less than 1 hour hi-vol operating time;
2. Usage of less than 75 percent of the asbestos aerosolized.

The resulting schedules can be seen in Table 3. Table 3 also represents the mass loadings and aerosol concentration in the chamber.

Table 3. Experimental Scheme for Simulated Air Samples.

Rum No.	Sample No.	Fiber Composition[a]	Sampler Type[b]	Filter Type[c]	Pore Size µm	Air Volume Filtered L	Sampling Time min.	Expected Mass Concentratoon of Fibers on Filter, µg/cm² Chrysotile	Amphibole	Fiberglass	Anticipated Mass Concentration of Fibers in Chamber µg/m³
1	1	C	P	M	0.22	32	16	1.385			Chrysotile 290
	2	C	P	M	0.45	32	16	1.385			
	3	C	P	M	0.80	32	16	1.385			
	4	C	Hi-vol	N	0.2	9116	14	6.503			
	5	C	Hi-vol	N	0.4	9200	13	6.563			
	6	C	Hi-vol	N	0.8	8400	11.2	5.993			
	7	C	P	M	0.22	512	256	22.161			
	8	C	P	M	0.45	512	256	22.161			
	9	C	P	M	0.80	512	256	22.161			
2	10	C + A	Hi-vol	M	0.22	2080	5.25	1.484	0.987		Chrysotile 290 Amphibole 193
	11	C + A	Hi-vol	M	0.45	2030	4.5	1.448	0.964		
	12	C + A	Hi-vol	M	0.80	2040	3.0	1.455	0.968		
	13	C + A	P	M	0.22	124	62	5.367	3.572		
	14	C + A	P	M	0.45	124	62	5.367	3.572		
	15	C + A	P	M	0.80	124	62	5.367	3.572		
	16	C + A	P	N	0.2	513	258	22.204	14.777		
	17	C + A	P	N	0.4	513	258	22.204	14.777		
	18	C + A	P	N	0.8	513	258	22.204	14.777		
3	19	C + A + F	P	N	0.2	7.6	3.8	1.406	0.401	0.201	Chrysotile 1240 Amphibole 354 Fiberglass 177
	20	C + A + F	P	N	0.4	7.6	3.8	1.406	0.401	0.201	
	21	C + A + F	P	N	0.8	7.6	3.8	1.406	0.401	0.201	
	22	C + A + F	P	M	0.22	29.2	14.6	5.404	1.543	0.771	
	23	C + A + F	P	M	0.45	29.2	14.6	5.404	1.543	0.771	
	24	C + A + F	P	M	0.80	29.2	14.6	5.404	1.543	0.771	
	25	C + A + F	Hi-vol	M	0.22	7920	20	24.159	6.897	3.448	
	26	C + A + F	Hi-vol	M	0.45	7701	17	23.491	6.706	3.353	
	27	C + A + F	Hi-vol	M	0.80	9167	13.5	27.963	7.983	3.992	

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[a] C = chrysotile
C + A = chrysotile + amosite
C + A + F = chrysotile + amosite + fiberglass

[b] P = Personal

[c] M = Millipore
N = Nuclepore

The XRF was used as an independent check on the asbestos mass determination. The results of the mass of chrysotile in a laboratory sample determined by both techniques were in excellent agreement. EM = 2.725 μg/m³ vs. 2.452 μg/m³ for XRF analysis.

1.4 Problems

There are several problems associated with using aerosol techniques to prepare airborne asbestos standards. The first limitation is that we used a liquid suspension to prepare the aerosol. It is not a fatal objection, but direct suspension of the fibers is a goal which would help assure minimal modification of the fibers.

Other difficulties in the experimental procedure are created by the broad range of fiber sizes present. The broad fiber size range imposes limitations and requirements on the equipment. The presence of the asbestos fibrils, which are numerous and weigh little, required very dilute suspensions and thus a long aerosolization time. On the other hand, the relatively huge amosite and fiber-glass fibers require much higher velocities to minimize fiber settling, and the loss of even a few of the largest significantly modify the mass concentration and mass ratios on the collected filter sample.

When using aerosol techniques to prepare asbestos filter standards, a laboratory is obligated to protect personnel and monitor both personnel and the environment in which the work is done. In addition, provision to exhaust the aerosol without adding asbestos to the ambient air must be made. These necessary precautions add to the time and cost of the preparation.

In our specific experiments, the health and emission problems were already solved, as the aerosol chamber used was isolated in an area ventilated through a HEPA cleaning system. The filter system was also piped to permit direct air purging of the chamber.

The design of the experiments was based on the specific goal of preparing filters of uniform loading in the fiber count ranges needed for the evaluation of Electron Microscopy Analysis of asbestos fibers. Thus the potential loss of large fibers affecting the precision and accuracy of mass estimates was accepted as an unavoidable risk.

During the development of the technique, it was learned that placing the hi-vol pumps, or at least their motors, directly in the chamber limited our available operating severely. A better solution to the problem would have been to bring the motors outside the chamber. This was considered, but due to time, cost, and the ability to the alternate short sample period to achieve our goals was not done.

The aerosol technique we used provided samples with primarily mono-dispersed fibers which could be collected on filters. The relative fiber loading and the mass loading were controllable to the extent required by the study. The reproducibility of the filter loadings was good, and the aerosol chamber was found to approximate a well-mixed reactor in its concentration behavior through time. Since our quality judgements were based on fiber dispersions and numerical loading, the uncertainty in the mass loading was not as critical a defect as it could have been.

If we were asked to reprepare standards using this basic procedure, the only significant change we would strongly recommend would be to aerosolize any amphibole asbestos fibers or glass fibers independently using an alternative generator of higher capacity. The result obtained with chrysotile fibers in the size range suspended in ambient air was quite satisfactory. The actual analyses were obtained using numerous preparatory and methodology variations, which makes direct comparison of the results meaningless.

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CONCENTRATION AND SEPARATION OF CHRYSOTILE BY TWO-PHASE LIQUID SEPARATION

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Abstract

The development of a rapid analytical method for determining chrysotile asbestos in water that requires substantially less time per analysis than electron microscopy methods will be described. Based on the proposition that separation of chrysotile from other waterborne particulate would greatly simplify the task of detection, the research effort was directed toward establishing separation and concentration techniques. This investigation led to the development of a separation procedure whereby chrysotile is extracted from a water sample into an immiscible organic liquid phase. The procedure is called two-phase liquid separation (TPLS).

TPLS extracts chrysotile from water into isooctane after the chrysotile surface has been rendered hydrophobic by reaction with an anionic surfactant (dioctyl sodium sulfosuccinate). Extraction of the chrysotile from the water phase into the isooctane phase occurs as the two liquids are shaken in a separatory funnel. Agitation creates an emulsion that is broken by adding sodium chloride solution. The isooctane is then filtered to deposit the chrysotile on a filter where its concentration is analyzed by light microscopy or spot test procedures.

During the course of work to develop a rapid analytical method for waterborne asbestos, a separation procedure for chrysotile was devised. This makes it possible to concentrate the chrysotile fraction and thus facilitate analysis by eliminating much of the interfering extraneous particulate. This method selectively extracts chrysotile from the water suspension into an immiscible organic liquid phase. The procedure is called two-phase liquid separation (TPLS).

Essentially, TPLS selectively extracts chrysotile from a water sample into a water-immiscible oil phase, such as mineral spirits, or isooctane, after the surface of chrysotile fibers is made hydrophobic through a reaction with an anionic surfactant. TPLS selectivity is based on the difference between the zeta potential of chrysotile and the zeta potentials of most other waterborne particulate. The surface charge of chrysotile is positive over a pH range from 2 to 11.4, whereas most other waterborne particulate is negative over this pH range. The chrysotile, therefore, selectively reacts with the negatively charged anionic surfactant, becomes hydrophobic, and is transferred from the water phase to the oil phase.

Extraction of the chrysotile from the water phase into the isooctane phase occurs as the two liquids are shaken in a separatory funnel. Agitation creates an emulsion that is broken by adding sodium chloride solution. The isooctane is then filtered to deposit the chrysotile on a filter.

The logic behind the approach taken in the development of a rapid analytical method was guided by an awareness of problems arising from gross amounts of interfering particulate prevalent in most water samples. The sample could be rid of interfering organic matter by low-temperature ashing, but the inorganic particulate could not easily be eliminated to leave only the asbestos. Analysis either had to be carried out in the presence of both organic and inorganic particulate or in the presence of inorganic particulate or the asbestos had to be separated from the interfering particulate. Analysis could be performed for separated and concentrated asbestos with much less difficulty than for asbestos in the presence of interfering particulate. Accordingly, our research effort emphasized the development of a separation method for asbestos in order to facilitate its subsequent detection. The separation of chrysotile was investigated first because chrysotile is the most prevalent form of asbestos found in most sampling locations. Also, its surface chemistry is sufficiently different than most other waterborne particulate to make its selective separation possible.

1. Effects of Critical TPLS Factors on Selectivity and Percent Recovery

Ths most critical factors in TPLS proved to be (1) mode of agitation, (2) type of surfactant, (3) concentration of surfactant, (4) number of extractions from a single water aliquot, (5) ratio of oil to water phase, (6) pH, and (7) the promotion of the reaction between the chrysotile surface and the surfactant. Although the effects of some factors overshadowed others, all were found to be important in the optimization of recovery and selectivity.

The problems of recovery and selectivity were dealt with separately during experimentation primarily because there was no satisfactory procedure to evaluate recovery when there were large amounts of interfering extraneous particulate in the original water suspension. Consequently, all recovery experiments were run on standard suspensions of pure chrysotile.

The general TPLS procedure in its stage of development at the beginning of these optimization studies consisted of adding anionic surfactant to the water phase, agitating the water phase and oil phase together, salting out the emulsion that formed upon agitation, and after phase separation, filtering the oil phase to concentrate the extracted chrysotile on a filter.

2. TPLS Recovery

Effects of Agitation. The mode of agitation is perhaps the most critical of all the investigated factors. When all other factors are optimized, if agitation is inadequate, low recoveries are inevitable.

First success was realized using ultrasonic treatment. Later, hand shaking in a separatory funnel produced good results. After consulting the literature, it was decided to attempt to obtain better control of agitation by substituting mechanical stirring for shaking by hand. Lai and Fuerstenau reported ~95 percent recovery of alumina from water suspension into isooctane using mechanical stirring and they were able to use it while varying other parameters to work toward optimum recovery. The effects of stirring appeared to be quite reproducible.

After switching to stirring, varying recoveries were obtained; some were quite high but others were low. This variability was attributed to other, possibly unidentified, parameters when the manipulation of identified parameters did not consistently improve results.

Mechanical stirring was performed at speeds of 600, 1200, and 2200 rpm and recovery was found to increase with stirring speed. However, stirring speeds of 2200 rpm and above were impractical. We settled on 1600 rpm, ran 18 experiments under identical conditions, and analyzed recovery by atomic absorption analysis for magnesium. A mean recovery of 28 percent ±14 was obtained. This degree of recovery was considered to be too low and to have unacceptable reproducibility. Consequently, a superior means of agitation was sought.

At this point, shaking by hand was tried again and better recoveries were found. A mechanical shaker was used and consistently higher recoveries were obtained than by stirring. Then differences were observed as factors other than agitation were varied. Ultimately, a recovery of 74 percent ±9.6 was obtained using shaking at 150 cycles per minute for five minutes. This was considered adequate for the purposes of the rapid analytical method.

Effect of pH. Hydrogen ion activity is not extremely critical in the range from pH 3 to 7, and there is no problem in maintaining the pH in this range during TPLS. However, to attain the highest positive charge on the chrysotile surface to promote maximum reaction with the anionic surfactant, the pH of the water phase was adjusted to 3.5 where the zeta potential has the highest positive value. This is accomplished usually by addition of 0.1 N HCl. In all cases, the pH of water samples has been found to be above 3.5.

Effects of Surfactant Compound and Surfactant Concentration. Only one type of surfactant has been investigated to any extent, this is the anionic alkyl sodium sulfosuccinate. However, two analogous compounds of this type have been investigated. Because of initial success with these compounds, dioctyl sodium sulfosuccinate (Aerosol OT and MO-70) and ditridecyl sodium sulfosuccinate (MT-70), no others were sought.

There was some evidence that MT-70 promoted slightly higher TPLS yields than Aerosol OT, but later experiments indicated comparable recoveries with MT-70 and MO-70, the dioctyl sodium sulfosuccinate supplied by Mona Industries, Inc., Paterson, New Jersey 07524. Greater TPLS selectivity was observed while using MO-70. Therefore, MO-70 has been recommended in the finalized TPLS procedure.

Surfactant concentration is critical in TPLS, not only from the standpoint of recovery but also in that too high concentration produces emulsion stability and phase inversion. Therefore, concentration must be carefully adjusted to obtain acceptable TPLS recovery and to avoid emulsification problems.

While employing MT-70, optimized recoveries were observed to occur at concentrations ranging from 1×10^{-3} M to 2.5×10^{-3} M. No phase inversion was detected at 1×10^{-3} M, but it was occasionally seen at the 2.5×10^{-3} M concentration. Recoveries were determined by AAS analysis for magnesium at these two surfactant concentrations to determine whether there was a significant difference in recovery. Twenty-nine determinations were made at 1×10^{-3} M MT-70 concentration and the recovery was measured to be 63 percent ±11. At 2.5×10^{-3} M concentration, it was measured to be 74 percent ±9.6.

Because no further problems with phase inversion were encountered, the higher surfactant concentration is recommended in the description of the TPLS method.

Ratio of Oil to Water Phase. The ratio of the volume of oil phase to the volume of water phase is important with respect to phase inversion. The oil volume was varied from 25 percent of the total liquid volume to 50 percent (1:1 water-oil). We finally settled on 100 mL of water phase and 50 mL of isooctane. Under these conditions satisfactory interfacial contact occurs and phase inversion is, in most cases, avoided.

Promotion of the Reaction Between Chrysotile and Surfactant. It was found that TPLS recovery was improved by heating the water phase after the addition of the surfactant. Presumably, heating promotes the reaction between the chrysotile surface and surfactant. Perhaps one of the mechanisms by which this occurs is the desorption of interfering species from the chrysotile surface.

Heating at 60 °C is recommended in the routine TPLS method.

3. TPLS Selectivity

Preliminary experiments to investigate TPLS selectivity were run on synthetic mixtures of chrysotile and diatomaceous earth. Diatomaceous earth was chosen because of the prevalence of diatoms in water samples. The recommended method is quite selective for chrysotile in the presence of diatoms.

It is impossible to predict the interferences that might be encountered when applying TPLS to water samples; however, TPLS has been run on water samples from 26 different sources. Some interferences were observed, but they were not extremely serious. All were related to lack of TPLS selectivity. Interferences that could give both high and low results were observed.

High results were obtained on some samples from which organic filaments were extracted by TPLS. Nevertheless, these filaments can be identified as not asbestos with a little experience. These filamentous structures are approximately 0.1 μm in diameter and have varying lengths. One of their identifying characteristics is that along their lengths they have swellings 0.2 μm diameter and approximately 0.5 μm long. These swellings can be seen in the light microscope with vertical illumination at 500x magnification after the filtered TPLS deposit has been carbon coated. No other interferences giving high results were discovered.

Two types of interferences produce low results. One is an extracted platy mineral that could obscure chrysotile fibers. These plates yield a diffraction pattern the same as that of talc. In no case was a sufficient quantity extracted to prevent the detection of chrysotile.

The other type of interference that will give low results is related to the nature of the chrysotile surface. If magnesium has been leached from the fiber surface, the zeta potential becomes negative, hence, it will not react with the surfactant. Also, coatings may form on the fiber and thus prevent access of the surfactant. Observed in the TEM, these coatings have low electron densities indicating that they are probably organic.

In the cases in which interference from organic material is a problem, heating after surfactant addition improves recoveries of coated fibers, but removal of organic fractions by a chemical treatment possibly would result in further improvement. Because of the time required, low-temperature ashing was not considered during the development of the rapid analytical method. Neither were "wet ashing" techniques explored. It is believed that any future work should include the investigation of "wet ashing".

4. TPLS Recovery as a Function of Particle Size and Aspect Ratio

Experiments were conducted to determine whether TPLS favors the extraction of certain chrysotile fiber sizes and aspect ratios. Several groups of experiments have been run investigating TPLS particle size recovery; however, the set of experiments that used the finalized TPLS conditions are the most pertinent. This last set of experiments also was designed to determine the effects on particle size recovery as a function of zeta potential.

Four samples of chrysotile having different zeta potentials were supplied by Mr. George Reimschussel of Johns Mansville Research and Engineering Center. Mr. Reimschussel measured their electrophoretic mobilities as listed below:

Sample	Electrophoretic Mobility at pH 10
96-1	+3.9 MV
96-2	+0.8 MV
96-3	-1.2 MV
96-4	-2.6 MV

No electrophoretic mobilities were measured at lower pH conditions. Nevertheless, they all have positive zeta potentials at pH 3.5-4. Good TPLS recoveries were obtained in this pH range. The existence of zeta potential variations among chrysotile types makes it important to control the pH during TPLS.

Standard chrysotile suspensions were prepared from samples 96-1, 96-2, 96-3, and 96-4, and equal (50 mL) aliquots were taken from the same suspension. One aliquot was merely filtered onto a 25-mm, 0.2-µm-pore-size Nuclepore filter; three other aliquots from the same suspension were subjected to TPLS and the extracted chrysotile was also deposited on the same type Nuclepore filters. Specimens for TEM were prepared from the filtered deposits by carbon coating followed by dissolution of the filter material by the Jaffe Wick Method.

Specimens were prepared for electron microscopy by carbon coating the filtered chrysotile deposits and dissolving away the Nuclepore filter by the Jaffe technique. TEM fiber counts and measurements of fiber lengths and widths were made of at least 100 fibers in each preparation. A computer was used to assign lengths, widths, and aspect ratios to size classes and to plot each versus cumulative number percent.

The results showed that TPLS favors the extraction of slightly longer fibers with slightly larger aspect ratios. In this respect, these results are consistent with those previously obtained under different TPLS conditions.

5. Summary of Results

Major findings were as follows:

(1) Percent chrysotile recovery by TPLS is optimized:
  ~ at pH 3.5 to 5.0
  ~ when the oil/water volume ratio is 1:2
  ~ when shaking is the mode of agitation
  ~ when 2.5 x 10^{-3} M anionic surfactant concentration is used
  ~ when five extractions are made from the same water aliquot.

(2) Seventy-five percent of chrysotile in water suspension can be extracted by TPLS.

(3) TPLS favors the extraction of longer fibers with larger aspect ratios.

(4) Filtration and resuspension is a practical means of concentrating particulate from large water volumes for TPLS. It is not as feasible to scale up TPLS to handle large water volumes as it is to filter and resuspend particulate in smaller, more manageable water volumes.

6. Steps in the TPLS Procedure

The following detailed procedure presents step-by-step the instructions for carrying out TPLS.

Step 1: Shake the bottom of water sample with a mechanical shaker for approximately five minutes at ~180 cycles per minute to create a uniform particulate suspension.

Step 2: Take an aliquot either directly from the water sample, or alternatively, filter an aliquot into a Metricel DM450 filter.

Alternative 2A: Take a 75-mL aliquot directly from the uniform water sample. adjust the pH to 3.5 ± 0.1 with 0.1 N HCl, add 25 mL of 0.01 M MO-70, heat to 140 °F while stirring, and allow to cool to room temperature. If acid additions overshoot the correct pH value, correct by adding 0.1 N NaOH. Heat at least to 140 °F; heating as high as the boiling point has no adverse effect except to extend the time required for cooling. Cooling may be accomplished by refrigeration or in an ice bath. Also, other than 75-mL aliquots may be taken; then the amount of surfactant added must be adjusted to maintain the 2.5×10^{-3} M concentration, and the amount of isooctane must be 1/3 of the total liquid volume.

Alternative 2B: Filter a relatively large aliquot (1 liter or more) of water samples to deposit the particulate on 47-mm Metricel DM450 (0.45 μm pore size) filter(s) (change filters if necessary as they become loaded to the degree that the water flow stops). Before the filter deposite have dried, ultrasonically remove the deposited particulate from the filter(s) in a beaker containing 75 mL of water to which 25 mL of 0.01 M MO-70 was added to make a total volume of 100 mL. Adjust the pH to 3.5 ± 0.1 with 0.1 N HCl, heat to 140 F, and allow to cool to room temperature.

Step 3: Combine the water phase from Step 2A or 2B with 50 mL of isooctane in a 250-mL separatory funnel equipped with a Teflon stopcock.

Step 4: Attach the separatory funnel to a mechanical shaker and shake at ~150 ± 25 cycles per minute for five minutes.

Step 5: Add 10 mL of 10 percent NaCl to break the emulsion. Shake lightly to distribute the NaCl in the emulsion. Occasionally the isooctane layer will form under the aqueous layer. Further gentle shaking will eliminate this condition.

Step 6: Remove the separatory funnel stopper during separation of the two liquid layers. Drain off the water phase through the stopcock. Allow time for the water droplets to settle and drain them off before pouring the isooctane from the top of the separatory funnel into the filtering apparatus. It is extremely important to take care that no water is filtered along with the isooctane.

Step 7: Deposit the separated chrysotile onto the filter by aspirating the isooctane through the filter.

Step 8: Combine the same water phase again with 50 mL of isooctane in the separatory funnel and repeat the extraction procedure without adding additional sodium chloride, and deposit the extracted chrysotile on the same filter. Repeat the extraction procedure three more times to make a total of five extractions from the same water aliquot.

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ANALYTICAL PROCEDURES AND STANDARDIZATION FOR ASBESTOS FIBER COUNTING IN AIR, WATER, AND SOLID SAMPLES

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Abstract

Interlaboratory analyses of air, water and mineral samples for asbestos fibers have shown much variability. Sources of error in this type of analysis include fiber losses or size modification during sample preparation, contamination by extraneous fibers, non-uniform deposition on analytical filters, differences between operators in fiber counting philosophy, and use of different criteria for fiber identification. The lack of suitable reliable standard samples has also confused efforts to incorporate good controls when analytical work has been split between several laboratories. Interlaboratory distribution of aqueous fiber dispersions for analysis has been found to be particularly difficult, and in several studies has resulted in a very wide range of reported concentrations from the same sample.

The published EPA interim procedures for determination of asbestos in air and water samples do not specify in detail the topics of fiber identification or fiber counting philosophy. Morphology, selected area electron diffraction, and energy dispersive x-ray analysis, used either separately or in combination can provide adequate fiber identification, depending on prior knowledge about the sample. However, economic considerations usually prevent classification of every fiber into its precise mineralogical species. A fiber classification system is proposed which provides a basis for uniform reporting of fiber counting data; some aspects of specimen preparation and fiber counting techniques area also discussed.

Key Words: Aqueous standard fiber dispersions; asbestos analysis variability; fiber identification criteria; interlaboratory calibration; preparation techniques.

1. Introduction

The control of asbestos in both the workplace and the environment requires reliable methods of measurement. The precise methods chosen depend upon the particular application. In the atmospheres of workplaces where asbestos is being processed or used, the membrane filter method [1][1], which incorporates fiber counting by phase contrast optical microscopy, can be used to provide an index of the airborne fiber concentrations. It is, however, important to recognize the limitations of this approach: the technique is non-specific in that all particles having aspect ratios exceeding 3:1, rather than just asbestos fibers, are counted, and the resolution is inadequate to detect fibers having diameters less than about 0.2 μm, whatever their lengths may be.

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[1] Figures in brackets indicate the literature references at the end of this paper.

Detection and identification of asbestos fibers in the environment outside of the workplace is a rather more difficult problem. A typical air or water sample contains a variety of minerals and fibrous organic debris from which asbestos fibers must be discriminated. Many minerals have good cleavage; elongated fragments of these will certainly be assigned as asbestos fibers in phase contrast fiber counts, and in some cases their precise identification may even present problems for electron microscopy techniques.

The transmission electron microscope (TEM) has been applied to measurements on environmental samples, using morphology, selected area electron diffraction (SAED) and energy dispersive x-ray analysis (EDXA) for fiber identification. Where an absolutely rigorous demonstration must be made of the precise mineral species, all of these techniques must be used, and analysis of electron diffraction data from several crystal orientations is required. Chrysotile is an exception to this rule in that the morphological characteristics combined with some supporting data by either SAED or EDXA are generally adequate for identification by experienced observers.

The scanning electron microscope (SEM) has limitations of both resolution and contrast [2], and fiber identification is confined to observations by EDXA of the chemical composition. The SEM-EDXA combination has therefore found little application to the identification and measurement of asbestos fibers in environmental samples, where there are numerous possibilities of confusion by other mineral species of similar chemical composition [3,4].

2. Sample Preparation Techniques for the TEM

The carbon-coated Nuclepore technique forms the basis of both the Environmental Protection Agency (EPA) interim procedure for water samples [5] and the EPA provisional method for air samples [6]. The steps in the technique are illustrated in figure 1. After collection of the sample on a Nuclepore filter a carbon film is applied by vacuum evaporation which envelops and traps all the particles on the filter surface. A portion of the coated filter is then placed on a support mesh (no carbon coating), and extracted in a Jaffe washer using chloroform as the solvent. The sample obtained is a copy, or replica, of the filter surface, with all of the original particles retained in position. The solvent extraction does not apparently cause loss of particles [2,7] since absence of a particle would usually be indicated by a replicated region with no particle inside it. The Nuclepore filter is selected for this technique, since its surface is relatively featureless apart from the cylinderical pores. Table 1 shows a summary of results obtained using this technique compared with those from other preparation methods. The filters analyzed were all prepared from identical loadings of the same fiber dispersion. It is evident that methods based on solvent washing of unfixed particles on membrane filters were all unsatisfactory, leading to large particle losses and non-uniform samples.

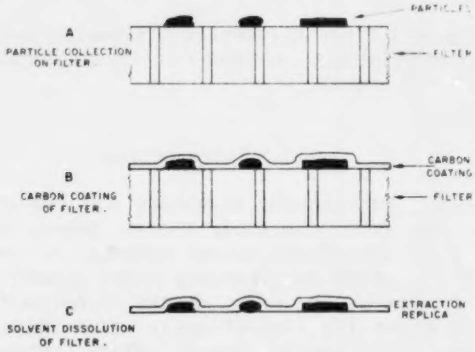
PARTICLES
A
PARTICLE COLLECTION ON FILTER
FILTER
CARBON COATING
B
CARBON COATING OF FILTER.
FILTER
C
SOLVENT DISSOLUTION OF FILTER.
EXTRACTION REPLICA

Figure 1. Steps in the carbon coated Nuclepore procedure for TEM specimen preparation.

Table 1. Comparison of TEM Sample Preparation Techniques

Chrysotile					
Preparation Technique	Number of Replicate Samples	Mean Value 10^6 Fibers/L	95% Conf. Interval 10^6 Fibers/L	Percentage of[a] Samples with Uniform Fiber Distribution	Fiber[b] Loss %
Carbon-coated 0.1 μm Nuclepore	26	23.4	20.0-26.9	85	-
Jaffe Washer Uncoated 0.1 μm Nuclepore	12	18.5	13.9-23.2	90	21
Jaffe Washer 0.45 μm Millipore	14	4.7	2.6- 6.8	50	80
Condensation Washer 0.45 μm Millipore	12	9.6	5.9-13.3	25	59
Jaffe Washer 0.22 μm Millipore	11	10.0	7.8-12.3	10	57
Ashing/ Coated Nuclepore	10	25.3	18.4-32.2	100	0

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[a] Filter uniformity demonstrated at 0.1 percent significance.

[b] Fiber loss calculated assuming carbon-coated Nuclepore technique to have zero loss.

Assuming that a transfer of the particulate from the original filter to the TEM specimen has been achieved without losses, and that the material has been retained in more or less the position it occupied on the original filter, errors in the measurement can occur in a number of different ways.

(a) The deposit may not have been uniform on the original filter.

(b) Contamination may have been introduced.

(c) Microscopists may differ in the way they count particular arrangements of fibers.

(d) Microscopists may accept different criteria for classification of fibers as asbestos.

In addition, depending on the type of sample, other errors may have been introduced at earlier stages in the preparation. For example, fibers on an air sample may have been moved or lost during transportation, and fibers in water samples may have flocculated or been scavenged from suspension by the container surfaces.

Uniformity of the filter deposit can be ensured by use of backing filters [7,8], to provide an even distribution of flow. However, these techniques cannot correct a basic aggregation or flocculation which may already be present prior to sampling. It has often been the practice to assume that fibers are deposited on the filter surface according to a Poissonian distribution, and to compute confidence intervals based on this assumption. Table 2 shows that particularly in the case of chrysotile in water, the assumption of a Poissonian distribution could not be justified, and that a normal distribution of larger standard deviation was a better fit to the data. Fundamentally, unless it can be demonstrated that there are no interactions between fibers during filtration, there is no reason to expect a Poissonian distribution. The Poissonian is the most favorable distribution which can be achieved at low filter loadings in ideal samples.

Table 2. Chrysotile in Water Samples: Goodness of Fit Test for Fiber Distribibition on Filters

	χ^2(Poisson)	χ^2(Normal)	Degrees of Freedom	Hypothesis Accepted
Chrysotile	1189	15.9	6	Normal
Crocidolite (total fibers)	32.5	6.8	3	Normal
Taconite (total fibers)	17.2	4.03	3	Normal
Crocidolite (SAED)	6.57	8.0	3	Either
Taconite (SAED)	1.53	1.78	3	Either

Contamination of samples by extraneous fibers has always been a problem in this type of analysis. Blank measurements are extremely important.

Fiber counting rules have not yet been defined precisely. This will not be possible until a decision has been made as to the treatment of fiber bundles and fiber aggregates. Unfortunately, particularly in the case of air samples, there are often many more fibers contained in a single loosely held aggregate than the total number of individual fibers that have been counted in the whole sample. The parallel fiber bundle is likely to be unopened fiber, and can be treated logically as a single fiber of larger width. The loose fiber aggregates can be dispersed in water using ultrasonic agitation, but if surfactants are added, chrysotile fiber bundles will also be dispersed into individual fibrils. The question of which measurement should be made, or which is biologically relevant, must be addressed before a solution to the fiber aggregate problem can be found. This aspect is extremely important, since some air samples collected on membrane filters are processed by ashing, ultrasonic redispersal in water and followed by the carbon-coated Nuclepore procedure. It would hardly be expected that these would yield the same results as those of the direct preparation, particularly if the fibers are highly aggregated prior to collection.

3. Other Preparation Techniques

There is only one current TEM preparation method which can be realistically compared in performance with the carbon-coated Nuclepore procedure. The "collapsing membrane" method [9] is illustrated in figure 2. Essentially the particulate is collected on a conventional mixed-esters sponge-type membrane, after which the filter structure is collapsed into a continuous film by exposure to solvent vapor. The filter is then carbon coated, and processed exactly as for the Nuclepore procedure, except that acetone is used as the solvent.

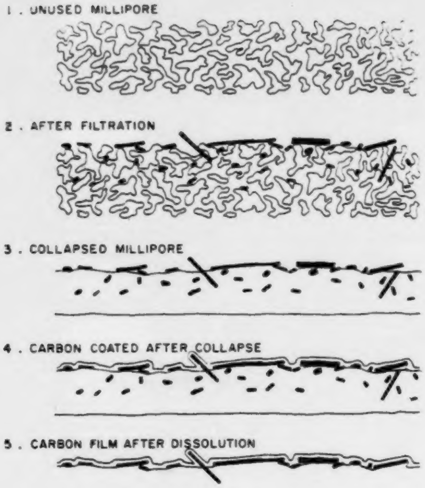
1 . UNUSED MILLIPORE
2 . AFTER FILTRATION
3 . COLLAPSED MILLIPORE
4 . CARBON COATED AFTER COLLAPSE
5 . CARBON FILM AFTER DISSOLUTION

Figure 2. Steps in the "collapsing Millipore" technique of filter preparation for the SEM and TEM.

Methods for selective concentration of asbestos fibers include ashing to remove organic debris [6,7,8] liquid density separation [10] and two phase liquid separation [11, 12]. More advanced techniques based on magnetic separation, ozone-UV oxidation [13] and high pressure oxygen treatment are under development.

4. Interlaboratory Calibration and Standardization

Interlaboratory calibration studies have been compromised by a number of factors, which are common to both air and water samples.

(a) No consistently reliable air or water standards have been available. In the case of air samples it is difficult to generate duplicate standards on which the fiber loadings are uniform. In the case of water standards it has been found difficult to produce a liquid standard dispersion which yields the same result after storage for even short periods of time.

(b) Until recently, different preparation techniques were in use in different laboratories.

(c) No systematic criteria have yet been established for counting of fiber bundles and aggregates.

(d) Analysts have differed in their interpretation of what criteria constitute satisfactory identification as asbestos.

Some progress can now be claimed in all of these areas, but (a) and (d) will be considered in detail.

5. Aqueous Standard Fiber Dispersions

It has been established that within a single laboratory, replicate filters prepared from the same dispersion of a single fiber type can yield replicate results using the carbon-coated Nuclepore procedure [7]. Figure 3 shows an example of the results obtained when ampoules of an aqueous dispersion of purified chrysotile in double distilled water were distributed to a number of laboratories experienced in asbestos fiber counting work. It is clear that in addition to an unacceptable degree of interlaboratory variability, there was also a significant trend towards lower concentrations when there were longer time intervals between issue of the samples and preparation of the filters. The results from the author's laboratory are identified, and these indicate that use of analytical

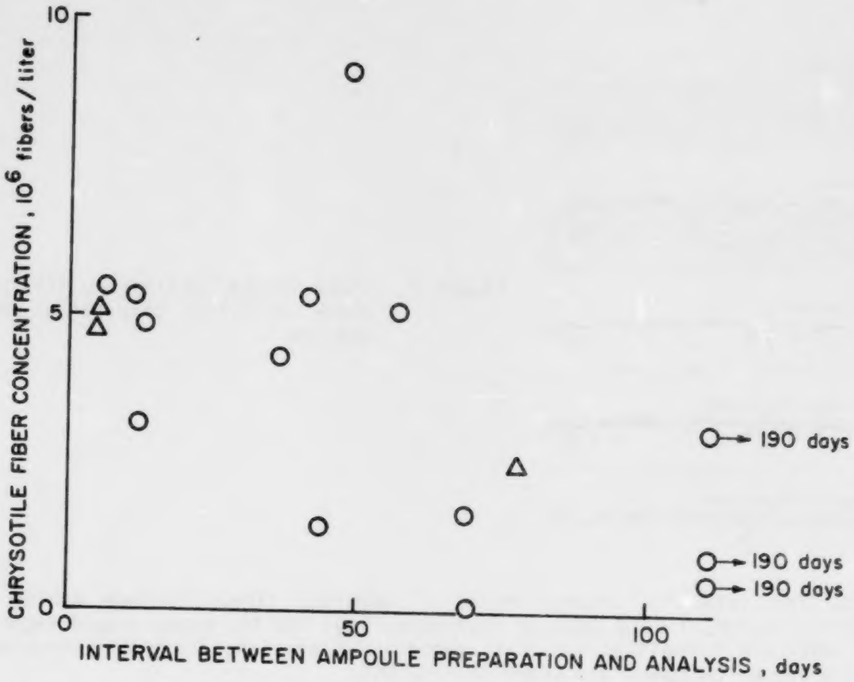

Figure 3. Results of interlaboratory analyses using aqueous dispersion of Union Carbide chrysotile fibers (Sample 11).

techniques identical in all respects did not allow reproduction of the initial fiber counts after an extended period of storage.

A large number of these ampoules were originally prepared, and they were studied more closely to determine the reasons for their failure as standards. Ampoules were opened and the contents filtered directly without the use of any ultrasonic treatment. No chrysotile fibers were detected on the filters, indicating that the chrysotile was no longer in suspension. The glass ampoule was cut open so that the interior surfaces could be gold coated and examined in the SEM. An interesting feature then emerged from this examination: many chrysotile asbestos fibers were found attached to the glass surface, and all of them were associated with some organic material of a gelatinous appearance. This effect is shown in figure 4. It was quite obvious that the asbestos fibers were cemented to the interior surfaces of the glass ampoule by this organic material, and that attempts to redisperse them using ultrasonic treatment of ampoules before they were opened were only partially successful. The origin of the organic material in these samples was at this point unclear.

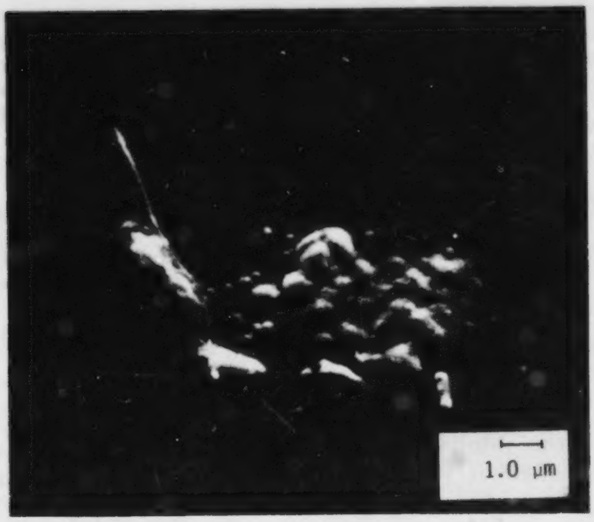

Figure 4. SEM micrograph showing chrysotile asbestos fibers attached by organic material to the inside surface of a glass container.

In a separate study, experiments on the dispersal of chrysotile in very dilute suspensions were initiated in order to assess the effects of pH and polyvalent ions which had been reported by Naumann and Dresher [14]. In the initial experiment, a chrysotile asbestos dispersion was divided into nine equal volumes in polyethylene bottles. Samples from three of these were filtered and analyzed immediately. The following treatments of samples prior to filtration and analysis were studied using two bottles for each treatment:

(a) continuous shaking in a laboratory shaker;

(b) continuous ultrasonic treatment in a bath; and

(c) static storage, followed by treatment for 15 minutes in an ultrasonic bath.

One bottle from each treatment was removed for filtration and analysis after 24 hours, and the second one after 7 days. The results are shown in Table 3. It is immediately evident that continuous shaking removed all of the fibers from suspension within 24 hours, whereas after 7 days in an ultrasonic bath the results were essentially unchanged. In static storage, followed by ultrasonic treatment for 15 minutes prior to filtration, the results fell by about a factor of 2 after 7 days. The curious and unexpected results for continuous shaking were thought to be due to collection of fibers by the inside surfaces of the containers, and re-suspension might be possible by ultrasonic treatment. The bottles which had been continuously shaken for 24 hours and 7 days were placed in an ultrasonic bath for a period of 30 minutes, and it was found that the re-suspended fiber concentrations were less than 10 percent of the initial value.

Table 3. Stability of Very Dilute Chrysotile Fiber Dispersions

(Fiber Concentrations in 10^6 Fibers/Liter)

	Treatment		
Time	Continuous Shaking	Continuous Ultrasonic	Static Storage with Routine Preparation
Initial	107	100	117
24 hours	<0.6	Not Analyzed	75
7 days	<0.6	78	51

Experiments were performed with polyethylene bottles containing equal volumes of the same dispersion, which contained approximately 3000 ng/liter of Union Carbide chrysotile.

Throughout this work, no particular effort had been made to maintain sterility in the suspensions, and it was at this point that the very important part which bacteria and their decomposition products play in the stability of dilute dispersions of chrysotile asbestos fiber was recognized. The importance of the electro-kinetic effects of chrysotile in removal of polysaccharides is well known in the beverage and pharmaceutical industry [15,16] and this lends support to the view that these organic materials may scavenge chrysotile fibers from suspension and adhere to the container walls.

A new dispersion of Union Carbide chrysotile was prepared, using sterilized glass containers and double-distilled water taken directly from the condenser outlet of a glass still. The dispersion was immediately transferred to 50 mL glass ampoules, and these were flame sealed. The ampoules were then autoclaved at 121 °C for 30 minutes in order to sterilize the contents. All of the preparation was completed within one day. Using this procedure no bacteria or their decomposition products should be present in the dispersion, and any organisms which may have accumulated during handling would be destroyed, so that no biological activity should exist in the final ampoules. Table 4 shows the results which have been obtained using 10 mL samples from these ampoules of the standard dispersion. It can be seen that even after storage for a period of 60 days, the fiber count remained unchanged, and that constant results were obtained without the use of ultrasonic treatment. The dispersions also showed no change after continuous shaking for periods up to 7 days. However, when an ampoule was opened and the contents contaminated with unsterile distilled water before being re-sealed, it was found that very variable fiber counting results were obtained after shaking. The nature and concentration of the organisms added, however, were not under good control. The results of similar work using UICC chrysotile are shown in Table 5. These sterile suspensions remained unchanged for long periods, but when they were contaminated with water containing active organisms the results were again unsatisfactory. In particular, the unsterile ampoule shaken for 7 days yielded a very inhomogeneous filter, and the chrysotile fibers were found to be strongly aggregated and attached to organic debris. In this case a large mass of organic material containing 20 fibers was found on one grid opening, resulting in a very large confidence interval for the calculated concentration value. Table 6 shows the results obtained to date using UICC crocidolite. Although this work is incomplete, it appears that stable dispersions of crocidolite can also be prepared.

Table 4. Stability and Storage of Sterile Union Carbide Chrysotile Asbestos Fiber Dispersions

(All Concentration Values in 10^6 Fibers/Liter)

	Mean	95% Confidence Interval
Analysis at initial ampoule preparation	82.6	44.5 - 121
After continuous shaking for 24 hours	73.5	47.1 - 99.9
After continuous shaking for 7 days	69.5	51.5 - 87.5
After 60 days storage, no ultrasonic treatment used	76.8 80.0	55.2 - 98.5 61.3 - 98.7
Unsterile ampoule shaken for 24 hours	29.4	15.0 - 43.8
Unsterile ampoule shaken for 7 days	16.7	4.4 - 29.0
Unsterile ampoule shaken for 4 days	62.4	38.8 - 86.4

Table 5. Stability and Storage of Sterile UICC Chrysotile Asbestos Fiber Dispersions
(All Concentration Values in 10^6 Fibers/Liter)

	Mean	95% Confidence Interval
Analysis at initial ampoule preparation	19.1	11.2 - 27.0
After continuous shaking for 24 hours	27.9	18.2 - 37.6
After continuous shaking for 7 days	25.1	15.9 - 34.3
After 52 days storage, no ultrasonic treatment used	24.8 22.4	12.5 - 37.0 15.9 - 28.9
Unsterile ampoule shaken for 24 hours	29.4	21.0 - 37.8
Unsterile ampoule shaken for 7 days	38.8	0 - 77.7
Unsterile ampoule shaken for 4 days	6.4	3.3 - 9.6

Table 6. Stability and Storage of Sterile UICC Crocidolite Fiber Dispersions
(All Concentration Values in 10^6 Fibers/Liter)

	Mean	95% Confidence Interval
Analysis at initial ampoule preparation	28.9	15.4 - 42.4
After 30 days storage, no ultrasonic treatment used	34.7 29.8	26.9 - 42.5 23.2 - 36.5

It has now become clear that much of the variability associated with interlaboratory analyses of liquid chrysotile fiber dispersions was due to the presence of biological organisms, the decomposition products of which scavenged the fibers and subsequently adhered to the surface of the containers. The result obtained was then a function of the extent to which this process had already occurred and the degree to which any ultrasonic treatment was able to detach and redisperse the fibers. If biological organisms are excluded, and absolute sterility is subsequently maintained, standard dispersions of chrysotile and crocidolite can now be prepared which appear to be stable for long periods of time.

6. Proposed Criteria for Identification of Asbestos Fibers

6.1 Chrysotile

Identification of chrysotile fibers can be achieved using various combinations of morphology, SAED and EDXA.

The scrolled structure of chrysotile gives rise to a characteristic morphology in the TEM, which is seen only in a few other minerals. This morphology is often referred to as "tubular", and in many cases observation of this may be an adequate identification. This internal morphology of the fiber is not seen in secondary electron SEM images. Although an experienced TEM operator can often distinguish chrysotile fibers from other confusing materials using morphological observations alone, the technique is too subjective for routine use without some other means of confirmation.

In the TEM, selected area electron diffraction is capable of precise identification of chrysotile fibers. Unfortunately, for reasons of instrumental variations, operator techniques, sample history, fiber crystallinity and size, a diffraction pattern on which a confident identification can be made is not always obtained. In many cases, no diffraction pattern is obtained at all. The frequency with which satisfactory SAED patterns are obtained from chrysotile fibers may be very low; cases are on record where one operator-instrument combination achieved 90 percent, where another obtained only 10 percent, using the same sample.

In those cases where a satisfactory SAED pattern is obtained, the identification is usually rather simple. Because of the scroll structure of the fiber, there is no requirement to tilt it to a precise angle. The pattern is always the same, and can usually be identified by inspection at the microscope. Moreover, bundles of parallel fibers give more or less the same pattern as that from single fibrils.

In the TEM the internal fiber morphology can be used as the primary identification, with the EDXA spectrum as the confirmatory technique. However, as in the case of SAED, there are a number of effects which may prevent satisfactory identification of an individual fiber. The magnesium-silicon ratio varies in fibers obtained from different sources, and subsequent treatment of the fibers is also capable of changing this ratio. Some of the magnesium is more loosely bound. In aqueous suspensions, and particularly if these are acidic, magnesium ions may be removed either partially or completely from the structure. Even in those cases where the magnesium has been completely removed, a silica shell remains which still has the characteristic internal morphology. The degree of dissolution of magnesium from chrysotile fibers in aqueous suspensions may be variable from fiber to fiber in the same sample. Acid leaching studies have shown that the magnesium-silicon ratio of some fibers remained unchanged after exposure to pH values of 3.0 for seven days, whereas others were not resistant in this way. Moreover, it has been found that a low magnesium content in an individual fiber is not necessarily related to an inability to obtain a satisfactory SAED pattern. Thus a fiber identified crystallographically as chrysotile by SAED may contain only a small proportion of the stoichiometric value of magnesium. The observation that fibers having the crystal structure of chrysotile may have a variable magnesium content indicates that a magnesium-silicon ratio approximately equivalent to the published composition cannot be used as an identification criterion for chrysotile.

Chrysotile Fiber Identification Criteria in the TEM

Fibers of chrysotile will be identified primarily by morphology, with supporting evidence from either SAED patterns or EDXA spectra obtained on a proportion of those fibers reported. Morphological characteristics required will be:

(a) the individual fibrils should have high aspect ratios exceeding 10:1 and be about 40 nm in diameter;

(b) there should be some evidence of internal structure suggesting a tubular appearance similar to that shown in figure 5;

(c) the electron scattering power of the fiber at 60-100 kV accelerating potential should be sufficiently low for internal structure to be visible.

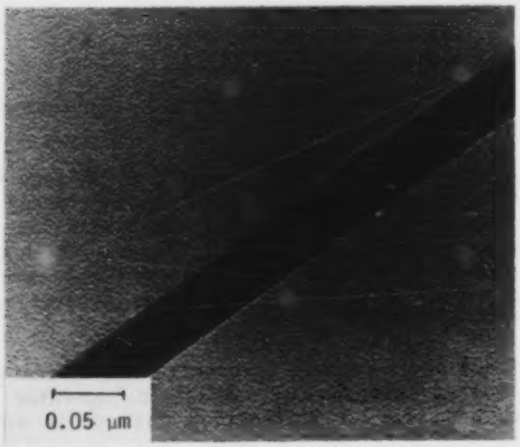

Figure 5. High magnification TEM micrograph of UICC Canadian chrysotile fiber. Note the symmetrical tubular appearance.

The fiber morphology should be inspected closely, since other minerals such as vermiculite exhibit scrolls (figure 6) which are similar in appearance.

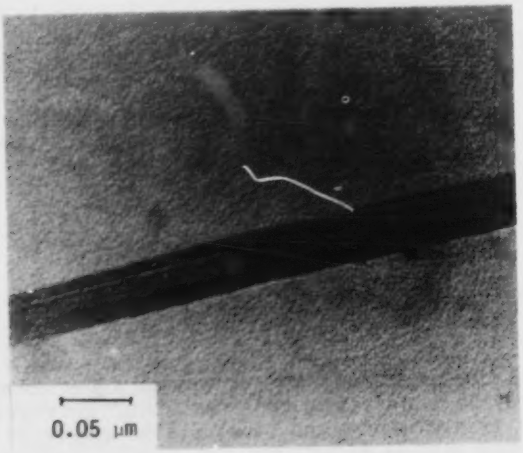

Figure 6. High magnification TEM micrograph showing scroll of vermiculite which could be incorrectly classified as chrysotile.

In order to report the presence of chrysotile, confirmation of the identity of typical fibers is required either by SAED or EDXA. The requirements for satisfactory identification by these techniques are discussed below.

SAED Confirmation of Identification. An SAED pattern should be obtained which appears similar to that shown in figure 7. The relevant criteria for identification of this pattern are indicated on figure 7. Most of these criteria can be viewed directly on the fluorescent screen of the microscope. Where a satisfactory pattern is obtained, identification can be made on the basis of visual observation only. However, a photographic record of a typical pattern should be made, which should also carry a gold ring calibration. The necessary identification criteria are as follows:

(a) a 7.3 Å spacing for the (002) reflections;

(b) a 5.3 Å spacing for the layer line repeat distance;

(c) a characteristic "streaking" of the (110) and (130) reflections.

A corresponding SAED pattern from scrolls of vermiculite is shown in figure 8. Although this might be classified as chrysotile in a cursory examination, it is clear that in addition to other relevant differences the 7.3 Å spacing is not present.

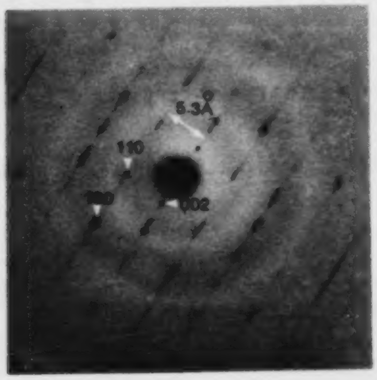

Figure 7. Selected Area Electron diffraction pattern from fiber of chrysotile asbestos. Identification should be made on the basis of 7.3 Å 002 spacings, streaking of the 110 and 130 reflections and a 5.3 Å layer line repeat spacing.

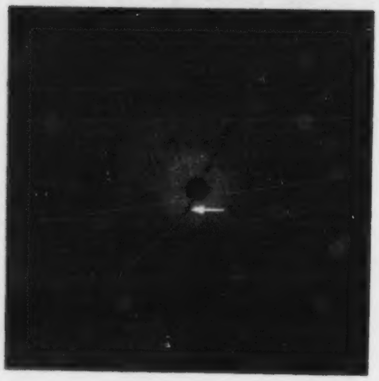

Figure 8. Selected area electron diffraction pattern from scrolls of vermiculite. Measurement of largest d spacing corresponds to 10.2 Å.

EDXA Confirmation of Identification. An EDXA spectrum from a typical fiber showing peaks from magnesium, silicon and iron only may be satisfactory confirmation of the presence of chrysotile. However, in many cases peaks from aluminum and calcium also appear in the spectrum, and the magnesium peak may be of variable magnitude for the reasons previously discussed. Confirmation by EDXA must therefore be on the basis of quantitative peak area ratios. Because of differences in detector efficiency and window thicknesses, the values of peak area ratios obtained from the same material will be different from instrument to instrument. A method of calibration is therefore required, and for simplicity this should be based on silicate samples of known composition. A substantial simplification of the procedure will result if these standards are selected to have compositions close to those of the asbestos varieties.

It is important to determine what values of the Mg/Si ratio should be accepted as confirmation of the presence of chrysotile. The work of Barbeau [17] indicates that two of the three magnesium ions in chrysotile are more easily removed by acids than is the third one. Dissolution of the third magnesium ion requires very drastic treatment. It seems possible that complete destruction of the crystal structure is not achieved until the third magnesium ion is attacked. Certainly, the variation of magnesium concentration from fiber to fiber observed in the TEM would support this view. Since it is now necessary to define a minimum magnesium concentration for classification of a fiber as chrysotile, it would be logical to establish a permissible range corresponding to more than one magnesium ion in the structure. The lowest permissible value could be provisionally set as 1/3 of the Mg/Si ratio obtained using UICC chrysotile.

The concentration of iron in chrysotile is not diagnostic, since a large proportion is in the form of associated magnetite. Limits on the Fe/Si, Ca/Si and Al/Si ratios must also be established, if other mineral fragments are to be excluded from the fiber count. Palygorskite, and particularly the Attapulgite variety, can be mistakenly classified as chrysotile on the basis of morphology and a superficial examination of the SAED pattern at the instrument. This mineral, however, can be discriminated from chrysotile by use of the Al/Si ratio, and also by careful measurements on the recorded SAED pattern.

As a provisional measure, it is suggested that the EDXA confirmation criteria should be as follows:

i) Mg/Si ratio greater than 1/3 of that from UICC chrysotile;

ii) Al/Si and Ca/Si ratios smaller than 0.05, unless it can be shown that the aluminum and calcium originate from extraneous particulate close to the fiber being analyzed;

iii) Fe/Si ratio not more than double that obtained from UICC chrysotile.

In a sample about which nothing is known, suspected chrysotile fibers can be classified as in Table 7.

Table 7. Classification of Fibers with Tubular Morphology

TM	Tubular morphology, similar to but insufficiently characteristic for classification as chrysotile
CM	Chrysotile by morphology only
CD	Chrysotile by SAED
CX	Chrysotile by EDXA
CDX	Chrysotile by both SAED and EDXA

6.2 Amphiboles

Unlike chrysotile, the amphiboles are of very varied chemical composition [18,19]. The lattice parameters are also variable within the species, depending on the composition. Amphibole is a very common constituent of the earth's crust and particles of it will be encountered frequently in environmental samples.

Amphibole has a pronounced cleavage parallel to (110). Because of this, crushed fragments of amphibole tend to be elongated, needle-shaped particles. These cleavage fragments are brittle and do not display the flexible fiber and strength characteristics of asbestos. Amphibole asbestos does not display the prominent (110) cleavage, and generally the fibers have higher aspect ratios than the cleavage fragments of the non-fibrous varieties. Studies on methods for discriminating between individual small particles of the fibrous and non-fibrous forms are in progress, but no simple method yet exists, apart from the observation that the cleavage fragment will often lie on a (110) face. It may be possible to distinguish a population of particles from a population of fibers on the basis of the distribution of aspect ratios. Proposals to increase the aspect ratio from the current 3:1 to 10:1 for definition of a fiber are based on the observation that the mean aspect ratio is much higher for fibers than for cleavage fragments. In the absence of any other method of discrimination, this proposal has some merit and is supported by many mineralogists.

The aspect ratio to be chosen should preferably have some mineralogical or biological relevance, rather than be another arbitrary value such as that currently in use. Unfortunately, any change from the current 3:1 value would receive substantial opposition from many legislative bodies, whose past records are based on this. Moreover, there seems to be inadequate evidence that cleavage fragments in the aspect ratio range of 3:1 to 10:1 do not have health effects similar to those of the fibrous mineral in the same aspect ratio range. The model of Pott [20] logically postulates that the carcinogenic potential is a continuous function of fiber size, and although the health effects of low aspect ratio fibers may be assigned in this model to be lower than those of the high aspect ratio fibers, they are not considered to be actually zero. In summary, a recommendation to increase the aspect ratio to 10:1 for definition of a fiber would currently have to be justified on the basis of analytical expediency alone. Selection of any other value between 3:1 and 10:1 would be an arbitrary compromise.

It is recognized that confidence in classification of a particle as a fibrous variety increases as the aspect ratio increases. The most convenient approach which is currently available to specify a fiber population to be either "asbestos" or "non-asbestos" is probably to examine the aspect ratio distribution according to the technique of Wylie [21].

Proposed Amphibole Identification Criteria

Amphibole particles and fibers viewed in the SEM or TEM display no characteristic morphology on which discrimination from most other minerals can be based. Morphological selection for further examination can only be made on the basis of aspect ratio, combined with observations of cleavage. Any additional characteristics such as evidence of flexibility and fibrillated ends add confidence to the classification as a fibrous mineral, and should be noted. Without any further identification, an individual elongated particle should be classified as an "unidentified mineral fiber". Because of the large amount of time required to perform an unequivocal identification, several degrees of identification will be defined in order to permit the analysis to be economically viable. It would obviously be desirable to identify each fiber completely, but this can only be achieved by some hours of labor expenditure on each fiber. Moreover, not all of the equipment or identification techniques may be available, and it is important that the method of identification be stated. However, if identification terminology is defined, there will be a minimum of confusion.

Terms for the identification techniques are defined below:

i) Routine SAED

SAED patterns obtained at random fiber orientations, without tilting of the specimen to align a crystallographic zone axis parallel to the microscope optic axis. Figure 9 shows a typical amphibole pattern obtained.

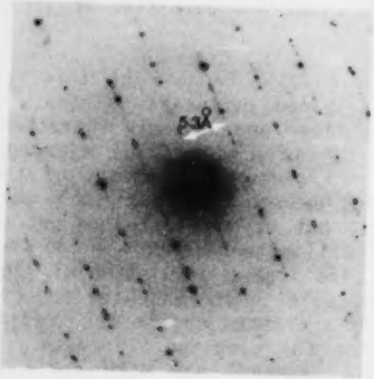

Figure 9. Amphibole SAED pattern (crocidolite) obtained from a fiber without precise orientation on to a zone axis.

ii) Routine EDXA

Examination of the EDXA spectrum for the presence or absence of peaks, without detailed quantitative interpretation.

iii) Zone Axis SAED

Precise tilting of the fiber using a goniometer stage to obtain an SAED pattern along a crystallographic zone axis. Dual Zone Axis SAED can also be performed, in which two different zone axis patterns are obtained from the fiber, and the angle between the patterns is also examined for consistency with the test amphibole structure. Some typical satisfactory zone axis patterns for this type of analyses are shown in figure 10. These also include rings from a thin film of gold as an internal calibration standard.

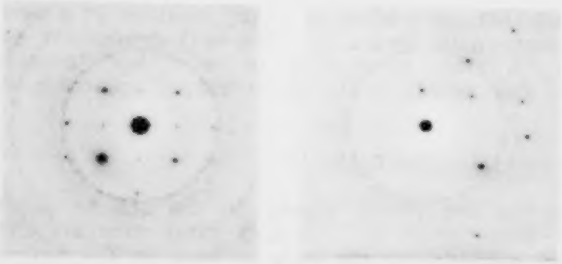

Figure 10. Selected area electron diffraction patterns from two different zone axes in anthophyllite.

Amphibole Fiber Identification Criteria in the TEM

As previously discussed, morphology should be used to select mineral fragments for further study. A search of the sample should be conducted of the sample for particles having aspect ratios greater than 3:1, with parallel or stepped sides. These should, in the absence of other background information, be classified as "unidentified mineral fibers". Obvious material of biological origin is excluded at this stage.

Further identification using either routine SAED or EDXA may not be possible. No recognizable SAED pattern may be obtained because of unsuitable fiber orientation. For example, it has been shown that only about 50 percent of amphibole fibers in random axial orientations yield a pattern which the operator can recognize as similar to that in figure 9. Using EDXA, the x-ray emission may for some reason not be able to reach the detector. In general, however, one or other of the techniques will be useful. The proposed classification scheme is shown in Table 8, for the *completely unknown sample situation*.

Table 8. Classification of Fibers Showing No "Tubular" Morphology

Identification Criteria	Code	Classification
Aspect ratio >73:1 and parallel or stepped sides	UF (Unidentified Mineral Fiber)	Unidentified mineral fiber (Suspected amphibole)
Routine SAED pattern obtained	AD (Amphibole by routine SAED)	Possible amphibole
EDXA spectrum obtained similar to that from standard amphibole asbestos samples	AX (Amphibole by routine EDXA)	Possible amphibole
Quantitative interpretation of EDXA spectrum, data available as mass percentages	AQX (Amphibole by quantitative EDXA)	Probable amphibole
Routine SAED *and* EDXA spectrum	ADX (Amphibole by both SAED and EDXA)	Probable amphibole
One zone axis SAED pattern consistent with amphibole, and EDXA spectrum	AZX (Amphibole by zone axis SAED and EDXA)	Confirmed amphibole
Two zone axis SAED patterns, consistent with amphibole, and angle between them is also consistent	AZZ (Amphibole by dual zone axis SAED)	Confirmed amphibole

In a sample about which nothing is known, the classifications in Table 8 are the most definitive identifications that can be made. The routine SAED pattern showing a 5.3 Å spacing shows only that a chain silicate may be present, or it may possibly be a layer silicate. Some clay minerals also give this spacing, e.g., palygorskite. Use of the routine SAED technique by itself is more a *rejection* technique than an actual identification, since obviously non-amphibole patterns are often obtained. Similarly, the EDXA spectrum showing a peak from silicon, with various combinations of peaks from sodium, magnesium, iron, and calcium is not sufficiently specific for identification of amphibole; a number of other minerals have these approximate compositions. Combined routine SAED and EDXA observations, or quantitative compositions calculated from EDXA spectra, give some additional confidence that the fiber is amphibole, but it is not absolutely certain. The use of zone axis SAED patterns, with computer analysis to determine their consistency with an amphibole structure, is the only way in which the presence of amphibole can be positively confirmed. Either one pattern with a routine EDXA spectrum, or two patterns with a known rotation between them along the fiber axis, should be the minimum criteria for positive confirmation. This also allows some discrimination to be made between individual amphiboles. Complete discrimination between the individual amphiboles requires matrix-corrected EDXA measurements so that accurate compositional data are available, in addition to the crystallographic data.

When amphibole is known to be present in the sample, either from previous knowledge of the source or from a zone axis SAED measurement, this gives much more confidence that the remaining morphologically and compositionally similar fibers initially classified as "possible" and "probable" amphibole are actually amphibole. The same situation applies if the sample being analyzed is one of a series from the same experiment or location, in which amphibole has been completely identified in some of the samples, and in which there is no reason to suspect a change of mineral type between samples. Under these circumstances the "possible" and "probable" categories should be reclassified as "confirmed amphibole". The unidentified mineral fibers, i.e., those which have not been rejected either by observation of an obviously non-amphibole SAED pattern, or by an obviously non-amphibole EDXA spectrum should then be reclassified as "probable amphibole".

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DEVELOPMENT OF A RAPID SURVEY TECHNIQUE FOR THE DETECTION OF ASBESTOS FIBERS

Peter Riis and Eric J. Chatfield

Ontario Research Foundation
Sheridan Park Research Community
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Abstract

Amphibole and chrysotile asbestos fibers adopt preferred alignment directions when suspended in a strong magnetic field. The alignment direction of the fiber may be parallel to, normal to, or at a specific angle to the flux lines. When an aligned distribution of fibers is illuminated by a collimated beam of light, scattering occurs in which maxima of intensity are observed in all directions normal to the fiber lengths.

The magnetic alignment and light scattering technique is being developed for the detection of asbestos fibers. The current procedure consists of filtration of a liquid sample in a magnetic field of 1.0 Tesla, during which the fibers become aligned before they come into contact with the filter. The mixed cellulose ester filter is mounted on a glass slide and its structure is collapsed by exposure to acetone vapor. The cleared membrane filter is removed from the slide and illuminated by a normal incidence laser beam. The scattered light intensity is measured as a function of the angular position of the original magnetic field direction, and the peaks which occur allow the concentration of aligned fibers to be deduced. This method shows much promise as a rapid survey technique for the presence of asbestos fibers in water samples. It is also possible to distinguish between chrysotile and amphibole asbestos fibers, and even between some specific amphibole varieties on the basis of differences in the profiles of their scattered light distributions.

Key Words: Asbestos fibers; magnetic alignment; magnetic filtration; light scattering; rapid fiber analysis.

1. Background

Current methods for the measurement of asbestos in water samples require the use of an electron microscope by a skilled operator for a time of typically three hours per sample. This procedure has the disadvantages of being slow, requiring expensive equipment, and it is also subject to operator subjectivity. A method which could be used to screen samples rapidly for asbestos content would be attractive. Thus if samples were shown to have asbestos concentrations below a specified threshold value they could be eliminated from further analysis. This would result in substantial economies in the effort required for monitoring of water supplies. Desired features of such a method would include the following:

(a) a requirement for substantially less labor than is demanded by current electron microscopy techniques;

(b) routine analysis without requirement for a high degree of skill;

(c) it should not require expensive equipment or a clean room environment;

(d) it should not rely on fiber counting techniques, either by electron microscopy or by light microscopy;

(e) it should be sufficiently sensitive to detect 2 x 10^5 fibers/liter or 1 nanogram/liter of asbestos;

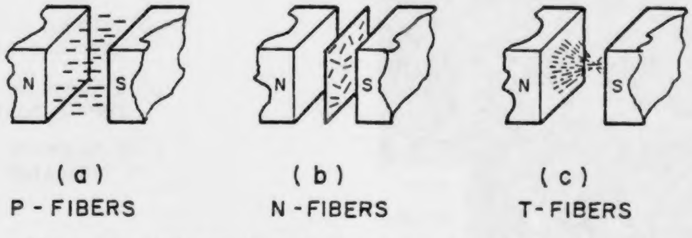
(f) it should be able to distinguish between chrysotile and amphibole asbestos.

This paper describes the results of progress to date by the Electron Optical Laboratory of the Ontario Research Foundation to develop such a method.

Previous attempts have been made to develop a rapid measurement technique for asbestos. Birks et al. [1][1] used electric fields to align chrysotile fibers and then measured the quantity by x-ray diffraction analysis. The sensitivity of the technique was 0.2 µg in the absence of other particulate. Melton et al. [2] developed the technique of two phase liquid separation for the separation of chrysotile asbestos from water samples. While this method removed other particulate giving a much cleaner sample, it still relied on light microscope counting techniques to obtain a 1 ng/L sensitivity. Another method developed by Diehl et al. [3] was based on single particle light scattering using a focused laser beam. This method is specific for distinguishing asbestos fibers from other particulate, but it requires a complete initial characterization of the water source by electron microscopy for calibration of the output. Any fluctuation in the relative proportions of different types of particulate may lead to an erroneous result. The possibility of error, and the requirement for prior TEM characterization of the sample make it unsuitable as a rapid screening technique. The approach being developed at ORF follows that described by Timbrell [4], namely the alignment of asbestos fibers by magnetic fields, followed by analysis of the scattered light from the aligned fibers.

2. Magnetic Alignment of Asbestos Fibers

When asbestos fibers are placed in strong magnetic fields (about 1.0 Tesla(T)) they become aligned in three possible modes as illustrated in figure 1. Depending on the type of fibers, and in some cases their origin, they may align parallel to the field direction (P-type), normal to field (N-type), or transversely to the field at a constant angle (T-type). Figure 2a is a phase contrast optical micrograph of a dispersion of Union Internationale Contre le Cancer (UICC) crocidolite; figure 2b shows the same dispersion but in this case the sample was prepared in a magnetic field of 1.0 T. In the case of crocidolite, the majority of the fibers align parallel to the magnetic field and a smaller number align in directions perpendicular to the field. Figure 2c shows a phase contrast optical micrograph of an aligned UICC amosite dispersion, which illustrates that for this material there is a greater proportion of N-type fibers. Figure 2d shows the alignment effect observed with UICC Canadian chrysotile. The fibers are P-type, but the fibers are curved, and there are larger deviations from precise alignment than is the case with the previous two materials. New Zealand cummingtonite which contains T-type fibers with their alignment directions symmetrically disposed about the field direction are shown in figure 2e.



(a) P-FIBERS (b) N-FIBERS (c) T-FIBERS

Figure 1. Alignment modes in air or liquid suspension.

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[1] Figures in brackets refer to the literature references at the end of this paper.

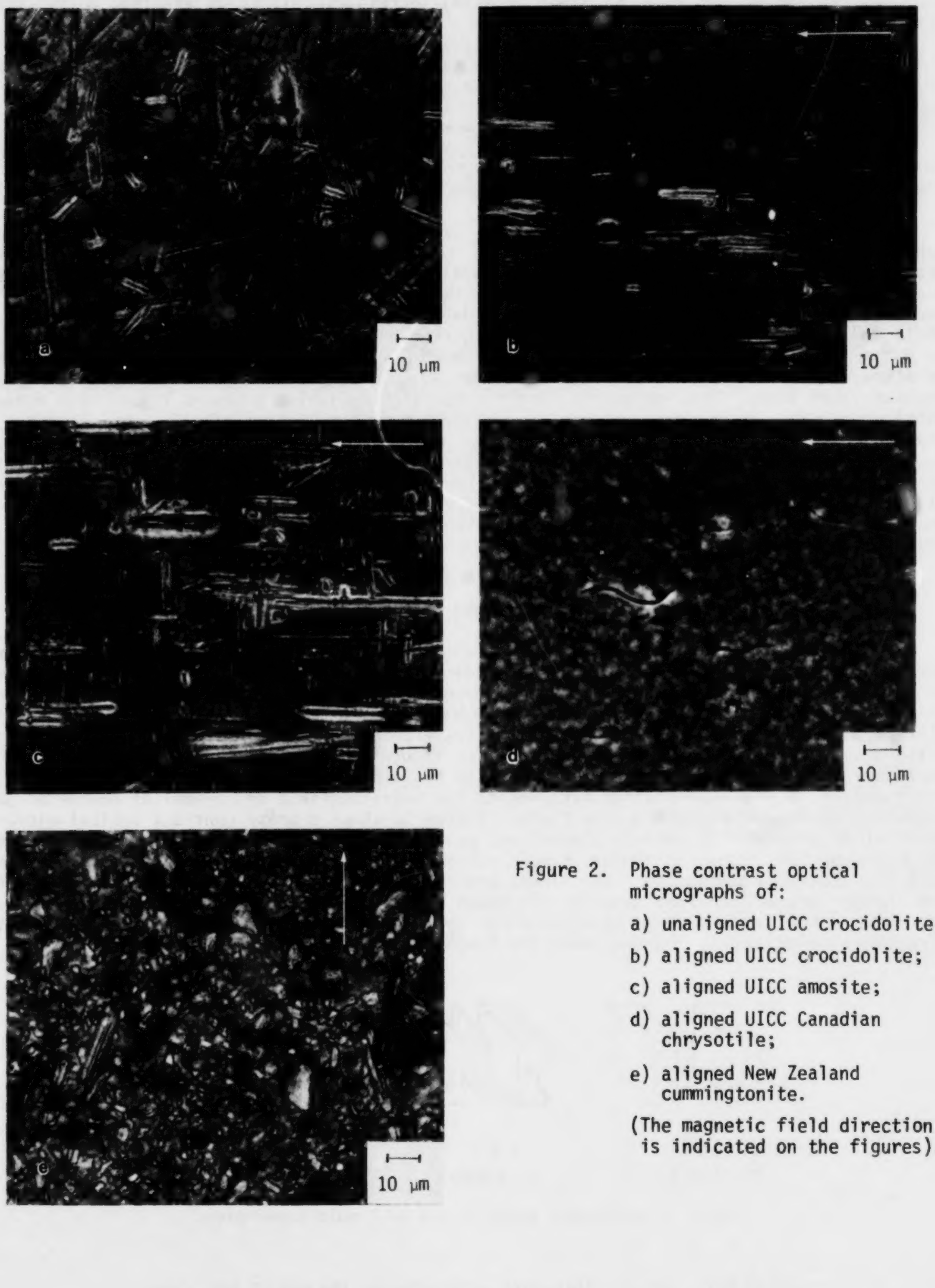
a
10 µm
b
10 µm
c
10 µm
d
10 µm
e
10 µm

Figure 2. Phase contrast optical micrographs of:

a) unaligned UICC crocidolite;

b) aligned UICC crocidolite;

c) aligned UICC amosite;

d) aligned UICC Canadian chrysotile;

e) aligned New Zealand cummingtonite.

(The magnetic field direction is indicated on the figures).

The alignment of asbestos fibers in magnetic fields is due to the paramagnetism or dilute ferromagnetism of the fibers. In amphiboles, the P-fiber alignment mode is a consequence of the direction of maximum magnetic susceptibility being parallel to the length of the fiber; for N-fibers it is normal to the length of the fibers and for T-fibers it is at a constant angle to the fiber length. It has also been suggested that the alignment behavior of iron-containing amphiboles is due to inclusions of magnetite [5]. Transmission electron microscope (TEM) examination of amosite fibers by Cressey and Whittaker [6] has not revealed any such inclusions. This suggests that the explanation of the alignment effect probably lies within the structure itself. They have studied the crystallographic orientation of aligned amphibole fibers by selected area electron diffraction. In the case of N-type UICC amosite fibers they found that the crystal $\underline{y}$-axis is oriented within ±20° of the field direction. This is explained if the axis of greatest magnetic susceptibility is parallel to the $\underline{y}$-axis and further supports the idea that the alignment effect is due to the crystallographic structure of the fiber. Angular restrictions about the z-axis of P-type fibers were also found but were of a much broader range. No explanation for the differences between P-type and N-type amosite fibers have so far been found. Analyses of their chemical compositions by energy dispersive x-ray analysis have shown no significant differences. In the case of chrysotile, Timbrell [4] has suggested that the fibers align in a direction parallel to the field because of magnetite particles in the fiber. This has not been investigated.

3. Preparation of Aligned Fiber Samples

A simple technique for the preparation of aligned fiber samples from aqueous suspensions is to add one percent by weight of agar to the dispersion and gently heat until the agar has dissolved. A small volume of the dispersion is then placed on the surface of a glass microscope slide which is located in a magnetic field of approximately 1.0 T. The liquid dispersion is left to solidify in the magnetic field, resulting in a permanent film which contains aligned asbestos fibers. This technique is useful for demonstration of the alignment effect, and was used for preparing the samples shown in figures 2 a, b, and c. It is not suitable, however, for the development of a rapid screening detection method since it requires the sample in the form of a highly concentrated dispersion.

The sample preparation method currently under investigation at ORF is based on "magnetic filtration". In this technique a 2.5 cm diameter glass Millipore filtration assembly is located between the poles of an electromagnet, and a non-magnetic clamp is used to attach the filter reservoir. The filtration apparatus is illustrated in figure 3. Fibers become aligned as the liquid passes through the magnetic field and when collected on the filter surface they retain their orientation. This technique has the advantage of allowing concentration of the fibers from a known volume of liquid onto the active area of the filter. The complete procedure is as follows.

(a) With a 0.22 µm pore size type GS Millipore filter mounted in the filtration assembly, the aspirator is turned on and the magnetic field adjusted to the desired value (typically 0.8 to 1.0 T).

(b) The desired volume of liquid is filtered through the assembly. For small volumes of liquid the filtration rate can be adjusted by changing the applied vacuum. A 20 mL volume of suspension is usually filtered in about two minutes. This ensures that the fibers have had adequate time to become aligned before contacting the filter surface.

(c) The magnetic field is then turned off.

(d) A mark is made on the edge of the filter nearest to one pole piece of the magnet. This provides an indication of the field direction after the filter has been removed.

(e) The filter is removed and dried for approximately 15 minutes at 70 °C.

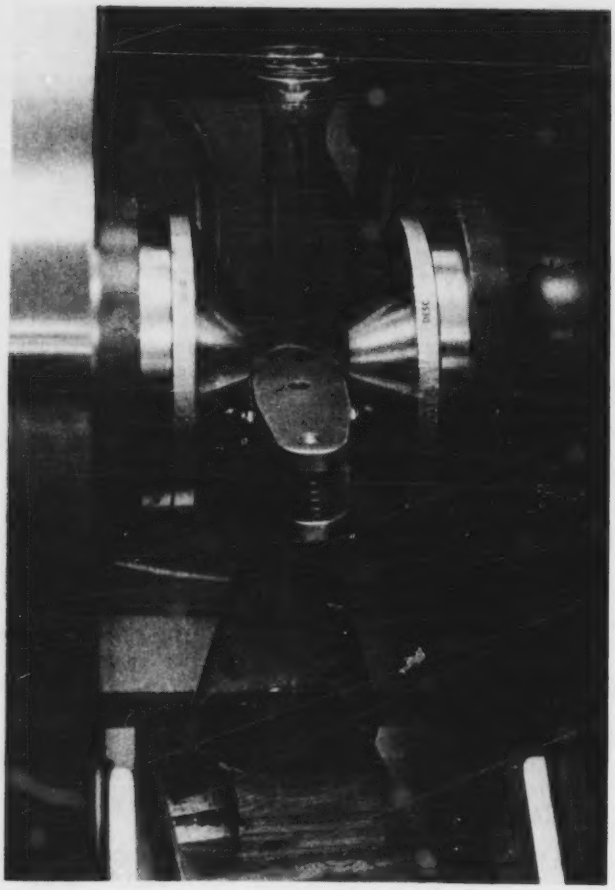

Figure 3. Equipment used for magnetic filtration.

(f) A clean glass microscope slide is used as a substrate for the filter. This is first dipped in a 20 percent solution of collodion in ethanol and allowed to stand for about 30 seconds, after which the surface film has become viscous. The filter is placed on to this surface film with a rolling action.

(g) The filter is then collapsed by exposure to acetone vapor, either by the Ortiz and Isom [7] technique or by that of the Asbestos International Association (AIA) [8].

If the sample is to be permanently mounted onto the slide, the edges of the filter should be sealed to the slide using clear nail polish or a similar lacquer. If the filter sample is to be used for scattered light analysis (described in the following section), the filter is carefully removed from the slide after cutting one edge to release it. This results in a sample consisting of a clear plastic membrane which contains aligned fibers, and which can be supported in a holder for scattered light analysis. Although the sample can be removed from the glass slide immediately after clearing, it generally peels from the surface easier if it is stored for about 24 hours after it has been mounted.

If aligned samples are to be prepared for TEM analysis, dispersions are magnetically filtered using 0.1 μm pore size Nuclepore filters, and TEM grids are prepared by the conventional carbon-coating Jaffe washer technique.

4. Analysis of Aligned Asbestos Fibers by Light Scattering

When a beam of light is used to illuminate a fiber, light is scattered perferentially in directions perpendicular to the length of the fiber. For a distribution of randomly-oriented fibers this results in random light scattering in all directions. This is illustrated in figure 4a, which shows the scattered light distribution for the unaligned dispersion of UICC crocidolite fibers of figure 2a. Figure 4a was obtained by photographing the scattered light when a laser beam was used to illuminate the sample. When a fiber distribution has been aligned in a magnetic field, the scattered light distributions from individual fibers are also aligned, and this results in a maximum in the scattered light intensity in directions perpendicular to the length of the fibers. This is illustrated in figures 4b to 4e for magnetically-aligned distributions of crocidolite, amosite, chrysotile, and New Zealand cummingtonite respectively. Most of the crocidolite fibers align parallel to the magnetic field, resulting in sharp scattering peaks perpendicular to the field direction, but small N-fiber peaks are also visible. Amosite contains large quantities of both P-type and N-type fibers, resulting in scattering maxima both perpendicular and parallel to the magnetic field. Chrysotile fibers align parallel to the field direction but because the fibers are often curved, there is incomplete alignment and the peak in the scattered intensity is much broader. New Zealand cummingtonite contains fibers which align at a constant angle to the field (T-type), giving rise to the "X" pattern of figure 4e.

Figure 5 shows schematically the equipment used for the analysis of the scattered light from aligned fibers. A beam of light from a laser is used to illuminate the aligned fiber dispersion. The fiber dispersion is rotated about an axis coincident with the center of the beam. A photomultiplier detector is mounted at an angle ϕ to the incident beam with its center of rotation at the center of the fiber dispersion. The angle ϕ can be varied. For a particular value of ϕ, the aligned fiber sample can be rotated through 360° (i.e.-can be varied from 0° to 360°). The detector output of scattered light intensity is then available as a function of the original magnetic field direction.

The actual instrumentation now in use is shown in figure 6. The laser beam of 514.5 nm wavelength from an argon ion laser is expanded to a diameter of approximately 1.5 cm. The diameter of the beam illuminating the sample can be varied using an iris. This arrangement allows a large area of the filter sample to be illuminated with light of constant intensity per unit area. The cleared filter membrane is mounted in a holder which is inserted at the center of the turntable. This is shown in figure 7. The scattered light is detected by a photomultiplier assembly mounted on an arm which can be rotated about the center of the membrane filter sample. The photomultiplier signal is displayed by an oscilloscope for initial evaluation of the samples, and by an x-y recorder which is used to obtain permanent records of the scattered light distributions. The sample turntable can be rotated at different speeds to accommodate this dual display technique.

Figures 8 a, b, and c show the outputs obtained respectively from crocidolite, amosite, and chrysotile dispersions. The quantity of asbestos fiber per square millimeter of filter area is indicated on the figures. These plots should be compared with the scattered light distributions shown in figure 4. Chrysotile has very broad output peaks, making it easily distinguishable from the scattered light outputs of the amphiboles. Furthermore the differences between amosite and crocidolite shows that differentiation between certain amphibole fiber types is possible.

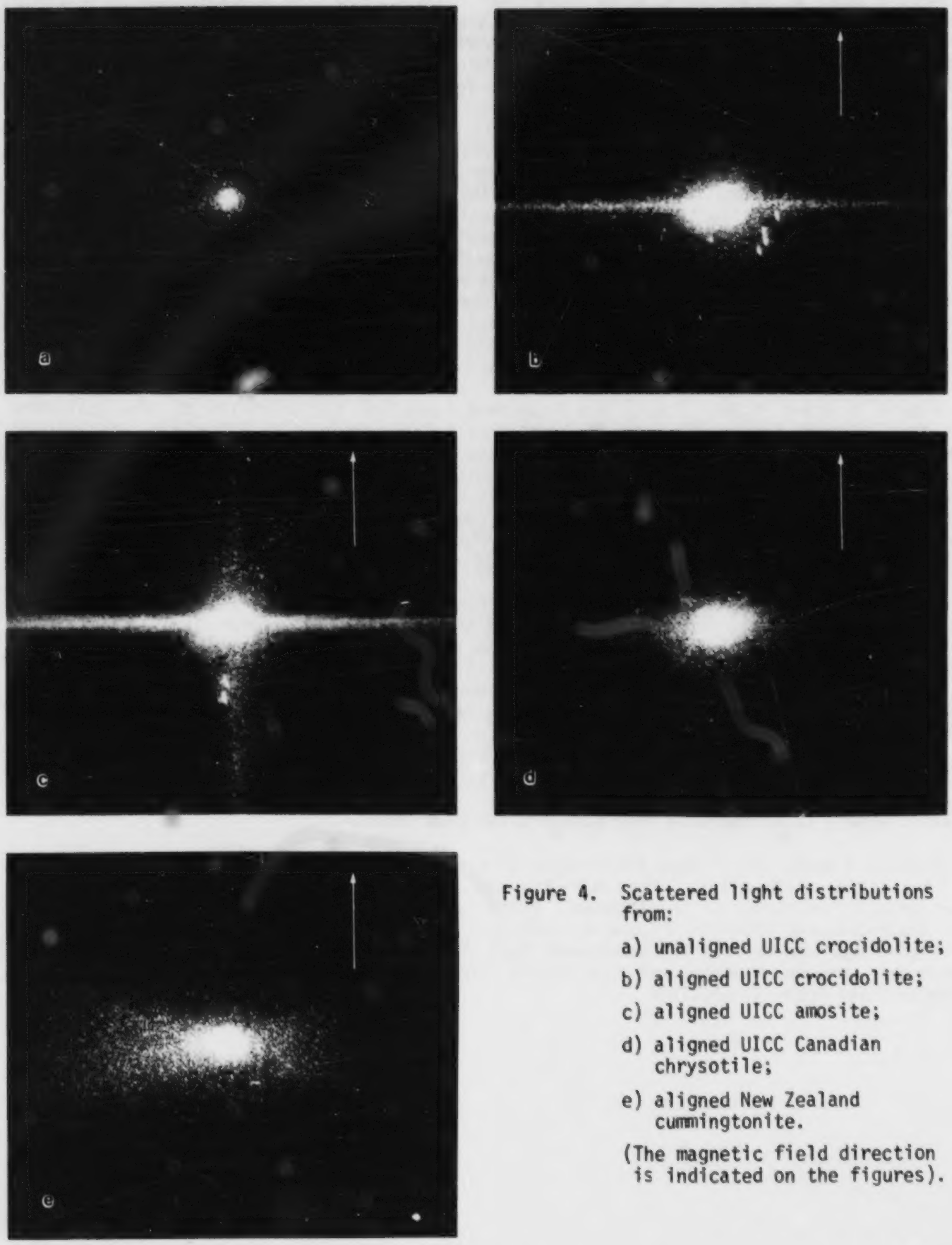

Figure 4. Scattered light distributions from:

a) unaligned UICC crocidolite;

b) aligned UICC crocidolite;

c) aligned UICC amosite;

d) aligned UICC Canadian chrysotile;

e) aligned New Zealand cummingtonite.

(The magnetic field direction is indicated on the figures).

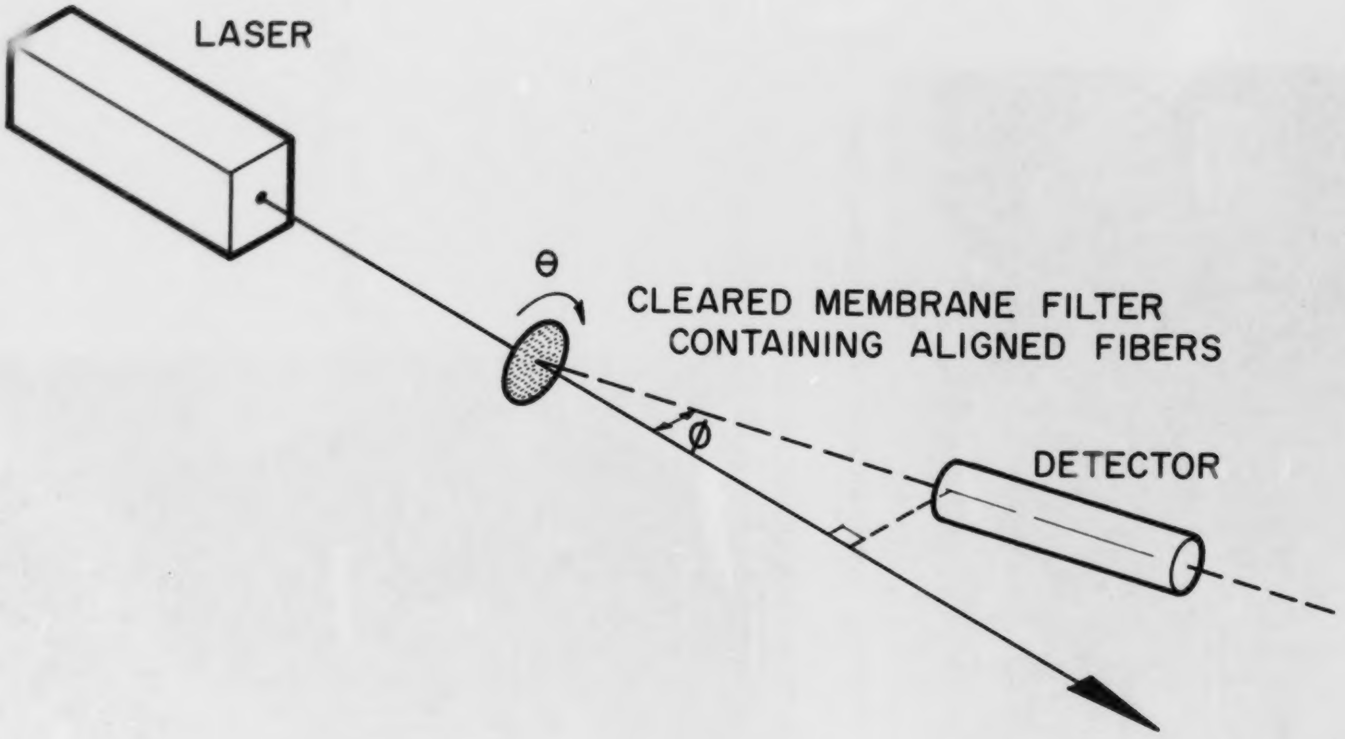
LASER
Θ
CLEARED MEMBRANE FILTER
CONTAINING ALIGNED FIBERS
φ
DETECTOR

Figure 5. Schematic of system used for the analysis of light scattered from aligned asbestos fibers.

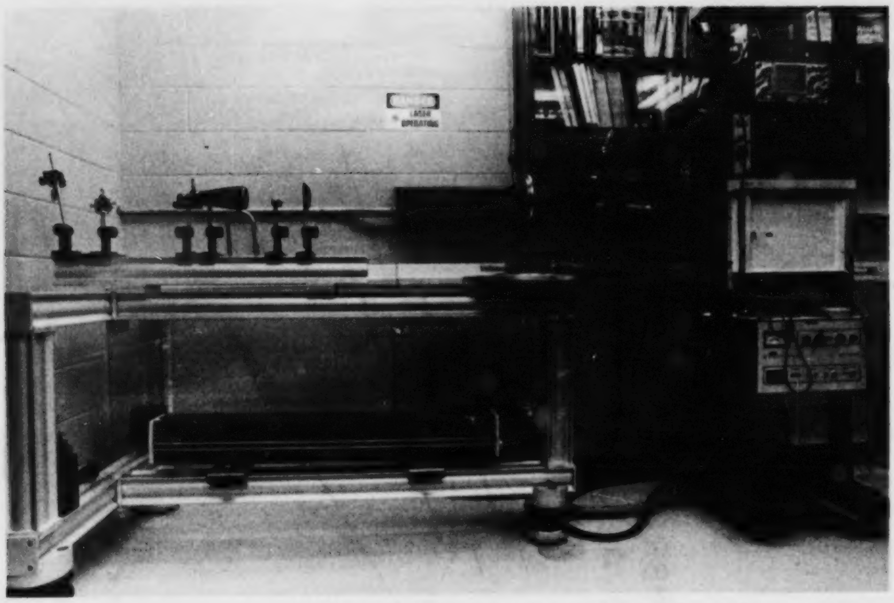
DANGER
LASER
OPERATING

Figure 6. System for light scattering measurements.

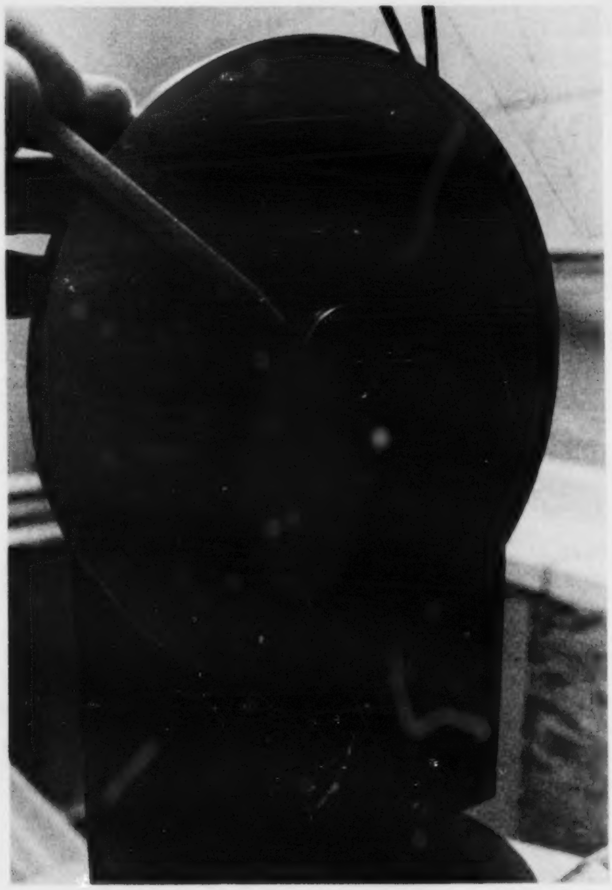

Figure 7. Filter holder for cleared membrane filters.

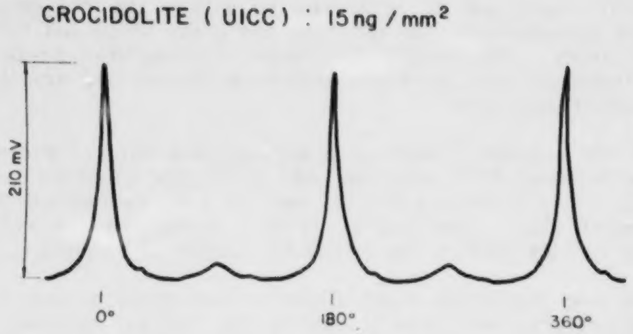
CROCIDOLITE (UICC) : 15 ng / mm²

Figure 8(a).

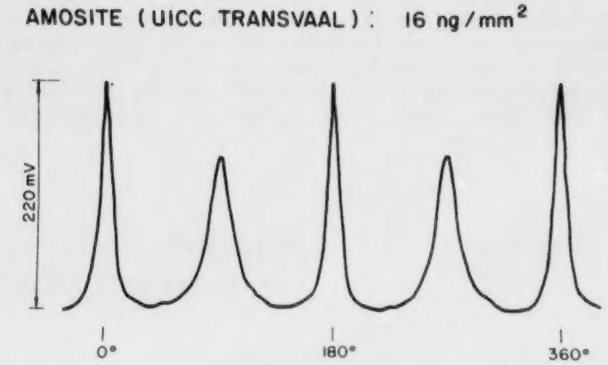
AMOSITE (UICC TRANSVAAL) : 16 ng / mm²

Figure 8(b).

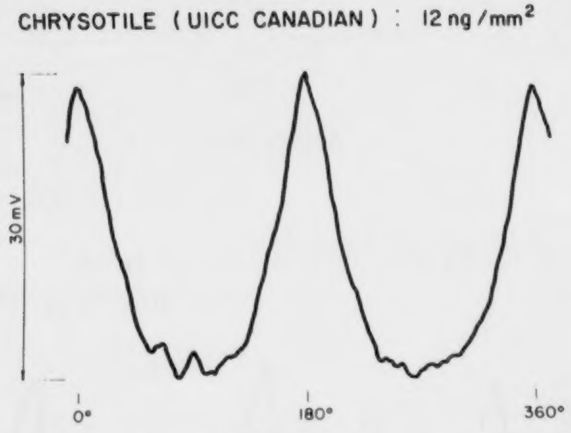
CHRYSOTILE (UICC CANADIAN) : 12 ng / mm²

Figure 8(c).

5. Results

Initial work was performed with cleared filter samples which were permanently mounted on glass microscope slides. In an effort to maximize the sensitivity of the method, possible noise sources were investigated. It was found that improperly cleaned glass slides resulted in substantial scattering, and even a cleaned blank slide had small symmetrical scattering peaks 180° apart which could be misinterpreted as being due to small concentrations of aligned fibers. Furthermore, samples mounted on slides showed spurious sharp scattering peaks which were due to scratches in either the collodion film or the glass slide. Samples were subsequently removed from the glass slide and followed by analysis of the cleared filter alone. The results no longer incorporated these spurious peaks, and the slide mounted technique was therefore abandoned in favor of examination of the cleared filter without the slide substrate.

Examination of the cleared filters by phase contrast optical microscopy has shown that the filter surface texture still remained when using the clearing technique described by Ortiz and Isom [7]. This structure would lead to some background scattered light which would limit the sensitivity. Clearing of filters using the AIA method [8] resulted in samples without this surface texture and is now the preferred technique.

Figure 9 shows the scattered light distributions from dilute dispersions for which distinct scattering patterns were obtained using crocidolite, amosite, and chrysotile. The chrysotile sample was sputter-coated with a gold film of approximately 100 nm thickness. This technique is currently under investigation as a means of enhancing the scattered light intensity from the filter samples. It is interesting to note that the volume of dispersion filtered for the crocidolite and amosite dispersions is 1/2 and 1/5 respectively of that which would have been used in preparing TEM samples to have approximately 10 fibers per grid square. Estimation of the detection limit as 1/10 of the peak height of these graphs would correspond to a detection limit of 0.02 ng of amosite per square millimeter of filter area illuminated by the laser beam, 0.04 ng/mm^2 for crocidolite, and 0.03 ng/mm^2 for chrysotile.

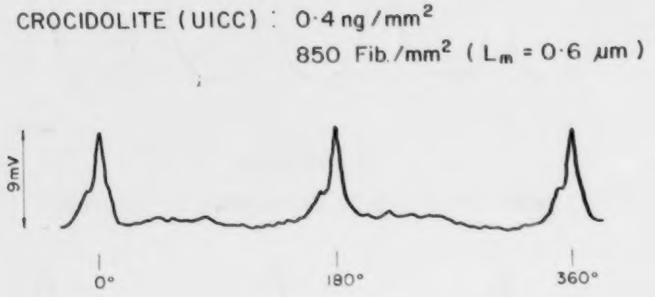

Figure 9(a).

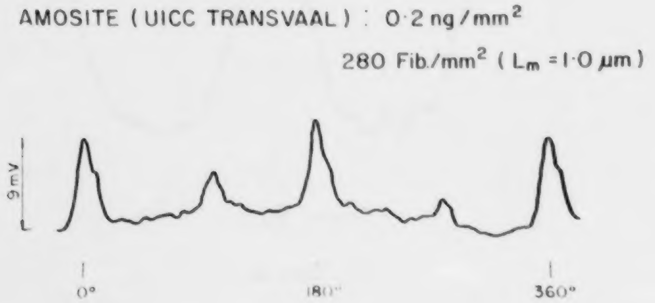

Figure 9(b).

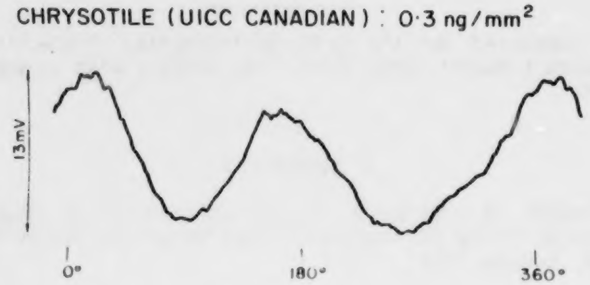

Figure 9(c).

To investigate the effects of non-fibrous particulate, various volumes of city water containing 0.3 μg/mL of total insoluble solids were mixed with 5 mL of aliquots of a 0.2 ng/mL dispersion of amosite, and the resultant dispersions were magnetically filtered. The effect of the non-fibrous particulate was to increase the value of the background intensity between the peaks on the plots. The sizes of the peaks remained approximately constant. Figure 10 shows the output for which the filter loading was approximately 50 ng/mm² of total insoluble solids and 1 ng/mm² of amosite. The experiments so far conducted indicate that approximately 0.1 percent by weight of amosite as a proportion of total insoluble solids was detectable.

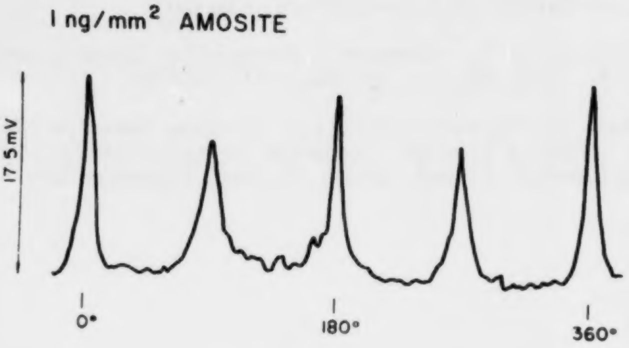

Figure 10.

6. Discussion

The current sensitivity of the magnetic filtration and light scattering technique is approximately 0.03 ng of asbestos fiber per square millimeter of filter area. Improvements in sample preparation technique and light scattering instrumentation are currently under investigation and it is likely that sensitivities will be further improved. The presence of non-fibrous particulate in a sample does not interfere with the detection of aligned asbestos fibers. Good reproducability has been obtained between samples containing the same concentration of amosite and a range of different concentrations of non-fibrous particulate. The sensitivity of the technique as an index of fiber concentration has also been demonstrated. In particular for three different filter loadings of amosite 0.2 ng/mm², 1.0 ng/mm², and 16 ng/mm², the corresponding peak heights were 9 mV, 17.5 mv, and 220 mV (figures 9b, 10, and 8b respectively). The experimental results show that magnetic alignment, combined with analysis of light scattering, shows great promise as a rapid screening method for asbestos detection.

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INTERLABORATORY MEASUREMENTS OF THE CHRYSOTILE ASBESTOS FIBER AND MASS CONCENTRATIONS IN WATER SAMPLES

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Abstract

In interlaboratory determinations of the chrysotile asbestos concentrations in water, it was found that:

1. The accuracy in the determination of mass in μg/L is about 50 percent, and is systematically low, i.e., the average recovery is 50 percent.
2. The precision in the determination of mass and fiber concentration is about ±50 percent.
3. The precision of the analyses did not improve during the three years of analyses involving six different chrysotile-containing samples.
4. Water samples retained in glass tend to produce lower mass determinations as the length of storage is increased.
5. Selected area electron diffraction provides highly variable results. In samples where the only fibers were chrysotile, the portion of fibers reported as to giving positive SAED patterns varied from 0-97 percent. The average fraction of chrysotile fibers with positive SAED for all investigators and investigations was 47 percent.

1. Introduction

Our initial work [1][1] in measuring asbestos concentrations in water showed that interlaboratory reproducibility was ±50 percent, using the analytical transmission electron microscope (ATEM). The earlier work involved the measurement of amphibole asbestos, whereas this study deals with the chrysotile form. Moreover, this report presents, for the first time, data on the accuracy of the ATEM method of measurement.

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[1] Figures in brackets indicate the literature references at the end of this paper.

A serious limitation of such interlaboratory comparisons involving complex equipment and technology is that some of the participating laboratories do not provide high quality data, primarily as a result of inexperience. This tends to bias the results negatively and to make the method appear less effective than it really is. Problems still exist in that uniform criteria for fiber identification have not been established. The laboratories, to varying degrees, base fiber identification on fiber morphology and/or fiber structure (using electron diffraction) and/or fiber chemistry (using energy dispersive spectrometry). In spite of these problems, it is clear that ATEM enables the asbestos content of water samples to be determined with accuracy and precision adquate for most environmental and toxicological studies being presently conducted.

2. Experimental Results and Discussion

In all published work, the precision of interlaboratory analyses has been reported. The ASTM Committee has attempted to determine the accuracy of the method in interlaboratory measurements, using clean water samples spiked with known quantities of chrysotile [2]. The results of this work are presented in Tables 1-4. Table 5 gives the results for an environmental sample, namely, water from a lake near a closed asbestos mine in Canada. The precision and accuracy achieved by the group are summarized in Tables 6 and 7 for the last six chrysotile samples studied by the group. The ASTM committee has analyzed seven chrysotile containing samples. The first group analysis is omitted from the present evaluation because of poor results [1] which were correctly attributed to a lack of experience with chrysotile. At the time of the original chrysotile analysis, most group members had worked only with amphibole asbestos.

Table 1. Results of Interlaboratory Measurement of Water Sample Spiked with 6.3 μg/Liter of Chrysotile.

	Fiber (mfl.)[a] Concentration	Mean Fiber Length μm	Mass in μg/ℓ	Percent[b] SAED
	700	1.76	45.8[c]	35
	918	1.69	10.9	70
	562	1.20	2.6	5
	510	1.29	3.7	30
	509	1.71	4.4	10
	1310	0.87	3.0	
	1100	1.26	9.5	30
	1040	1.38	4.9	15
	1290	1.31	3.0	55
	830	1.70	4.6	
Mean	877	1.42	5.2	31
± 1σ	±305	±0.3	±3.0	±22

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[a] mfl - means millions of Fibers/Litre.

[b] Percentage of chrysotile fibers giving positive selected area electron diffraction.

[c] Excluded from mean.

Table 2. Results of Interlaboratory Measurement of Water Sample Spiked with 0.63 μg/Liter of Chrysotile.

	Fiber (mfl.)[a] Concentration	Mean Fiber Length μm	Mass in μg/ℓ	Percent[b] SAED
	30	1.35	0.21	75
	29	1.08	0.15	97
	22	1.09	0.12	5
	23	1.21	0.13	26
	29	1.28	0.18	29
	27	1.41	0.63	30
	22	0.96	0.07	15
	51	1.38	0.13	53
	22	1.21	0.08	
Mean	28	1.22	0.19	41
± 1σ	±9	±.15	±.17	±31

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[a] mfl - means millions of Fibers/Litre.

[b] Percentage of chrysotile fibers giving positive selected area electron diffraction.

Table 3. Results of Interlaboratory Measurement of Water Sample Spiked with 0.127 μg/Liter of Chrysotile.

	Fiber (mfl.)[a] Concentration	Mean Fiber Length μm	Mass in μg/ℓ	Percent[b] SAED
	5.3	0.94	0.084	40
	4.3	0.77	0.032	88
	4.8	1.60	0.120	90
	9.1	1.30		10
	14.0	1.10	0.046	
	8.6	1.30	0.026	
	1.4	1.06	0.106	13
	1.6	0.54	0.004[c]	
	4.7	2.10	0.110	36
	5.0	1.67	0.066	20
	5.3	1.47	0.032	26
	5.4	1.24	0.041	65
	3.1	1.72	0.036	69
	0.2		0.002[c]	50
	5.0	1.25	0.029	60
	6.0		0.085	75
Mean	5.9	1.3	0.061	49
± 1σ	±3.0	±0.4	±0.034	±28

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[a] mfl - means millions of Fibers/Litre.

[b] Percentage of chrysotile fibers giving positive selected area electron diffraction.

[c] Excluded from mean (delayed filtration).

Table 4. Results of Interlaboratory Measurement of Water Sample Spiked with 0.56 μg/Liter of Chrysotile.

	Fiber (mfl.)[a] Concentration	Mean Fiber Length μm	Mass in μg/ℓ	Percent[b] SAED
	40	1.00	0.40	40
	13	1.13	0.17	93
	12	1.9	0.36	74
	15	2.1		0
	9	1.4	0.15	38
	3	2.3	0.03[c]	
	19	1.71	0.31	84
	29	1.94	0.47	34
	24	1.97	0.53	41
	14	0.92	0.13	50
	10	1.25	0.088	80
	6	1.34	0.075	65
	<1		0.099[c]	91
	17	2.45	0.13	50
	23		0.21	88
Mean	18	1.6	0.25	59
± 1σ	±9	±0.5	±0.16	±27

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[a] mfl - means millions of Fibers/Litre.

[b] Percentage of chrysotile fibers giving positive selected area electron diffraction.

[c] Excluded from mean (delayed filtration).

Table 5. Results of Interlaboratory Measurements for an Environmental Sample from a Lake in Canada Near a Closed Asbestos Mine.

	Fiber[a] Concentration	Mean Fiber Length μm	Mass in μg/ℓ	Percent[b] SAED
	66	0.83	0.20	35
	50	1.46	2.06	96
	7	1.40	0.05[c]	72
	52	1.60		2
	32			
	12	1.62	0.17[c]	
	69	1.05	0.70	26
	75	1.54	1.70	39
	111	0.75	0.54	28
	41	1.19	0.43	
	9	1.78	0.16	18
	<1	1.75	0.04[c]	70
	17	1.75	0.09	15
	56		0.47	64
Mean	53	1.3	0.7	42
± 1σ	±29	±0.4	±0.7	±29

---

[a] mfl - means millions of Fibers/Litre.

[b] Percentage of chrysotile fibers giving positive selected area electron diffraction.

[c] Excluded from mean (delayed filtration).

Table 6. Interlaboratory Accuracy for Clean Water Samples Spiked with a Known Quantity of Chrysotile Asbestos.

Sample: Added Chrysotile in µg/ℓ	Measured Mass in µg/ℓ Group Mean ± 1σ	Accuracy $\frac{\text{Measured-Added Mass}}{\text{Added Mass}}$ in %	Recovery in %	Measured Mass in µg/ℓ-Calculated using d = 415Å group mean ± 1σ
6.3	5.2 ± 3.0	- 17	83	5.1 ± 1.6
0.63	0.19 ± 0.17	- 70	30	0.15 ± 0.05
0.127	$0.06_1 \pm 0.03_4$	- 52	48	
0.56	0.25 ± 0.16	- 55	45	
Mean ± 1σ		48 ± 22	52 ± 22	

Table 7. Compilation of Precision and Accuracy Data for Six Samples Containing Chrysotile Asbestos.

	Mass Determination			Concentration Determination		
Sample: Added Chrysotile in µg/ℓ	Measured Mass µg/ℓ Mean ±1σ	Accuracy in %	Precision ±σ/mean Relative σ	Concentration in MFL: 10^6 Fibers/liter Mean ±1σ	Precision ±σ/mean Relative σ	Percent of Fibers Giving Positive SAED: Mean ±1σ (range)
6.3	5.2 ± 3.0	- 17	58, 31[a]	877 ± 305	35	31 ± 22 (5 - 70)
0.63	0.19 ± 0.17	- 70	89, 33[a]	28 ± 9	32	41 ± 31 (5 - 97)
0.127	$0.06_1 \pm 0.03_4$	- 52	56	6 ± 3	50	49 ± 28 (10 - 90)
0.56	0.25 ± 0.16	- 55	64	18 ± 9	50	59 ± 27 (0 - 93)
Canadian Lake Sample	0.7 ± 0.7	unknown not spiked	100	53 ± 29	55	42 ± 29 (2 - 96)
12/6/76	not reported	not measured		59 ± 24	41	not reported
Mean ±1σ		48 ± 22	46 ± 17[b]		44 ± 9	

---

[a] Mass based on fibril concentration and fibril diameter of 415 Å.

[b] For spiked samples only using footnote a values.

The results in Table 6 show that the measured mass was always less than the added mass, with recoveries ranging from 30 to 83 percent and averaging about 50 percent. The accuracy, defined as 100 (measured mass − true mass)/true mass, ranged from 17 to 70 percent and averaged about 50 percent. The actual accuracy is probably somewhat better than this because of the loss of fibers associated with delays in filtration. Figure 1 is a plot of the mass determined for the 0.127 µg/L sample as a function of the days of storage prior to filtration. It is apparent, notwithstanding the scatter, that the measured mass tends to decrease after long storage times. The 0.63 µg/L sample was prepared from an aliquot of the 6.3 µg/L sample and was filtered after storage. This may account for the poor accuracy (70%) in the case of the 0.63 µg/L sample.

A significant problem in reporting mass is the need to measure the fiber diameter. This task cannot be accomplished by visual determination on the fluorescent screen of the microscope. A careful determination of the fibril diameter characteristic of the addition was made by one laboratory and found to be 415 Å. Using this value and reported fibril (not fiber) concentrations to calculate the measured mass does not significantly alter the mean value of the reported mass. However, it does markedly reduce the scatter, i.e., it improves the precision (see Table 6, column 5).

Table 7 summarizes the precision and accuracy values for the chrysotile samples. From these limited data, it appears that the precision in the determination of fiber concentration and chrysotile mass is about ±50 percent. The accuracy is also about 50 percent and is systematically low. There does not appear to be a significant correlation between accuracy or precision and sample mass or fiber concentration within the range examined. Thus, the precision and accuracy of the ATEM technique remains at about 50 percent for the determination of chrysotile asbestos in water. There was no improvement during the 3 years of analysis (August 1976 to December 1979), as shown in figure 2. These results were achieved on relatively clean water samples, i.e., spiked samples and lake samples. The interlaboratory reproducibility would not be as good on relatively unclean water samples, such as plant effluents or most rivers. On the other hand, if the only data included in the evaluation were from laboratories making measurements routinely under carefully controlled conditions, the interlaboratory reproducibility would be improved.

There are many reasons for what appears to be mediocre precision and accuracy and the reliance on electron diffraction is a major contributor to the problem. In these samples, where the fibers were known to be chrysotile, only 47 ± 27 percent of the fibers gave identifiable selected area electron diffraction (SAED) patterns, and the percentage of fibers so identified varied greatly between the different laboratories (0-97%). This is primarily due to differences in equipment and to the criteria used to classify a SAED pattern as positive. Improvement in the diffraction portion of the analysis has been reported [3].

Refinements and improvements in the methodology are being pursued and these will undoubtedly lead to better interlaboratory comparisons. In the meantime, accuracy and precision of 50 percent would appear to be sufficient to address any meaningful water contamination problem involving asbestos.

The Committee has studied some problems associated with the analysis on a limited basis and found that leaching of MgO from chrysotile is rapid in HCl, but does not occur after 7 days in 30 percent NaOH [4]. The fiber contamination of new Nuclepore and Millipore filters and the fiber loss associated with storage in glass are still under investigation.

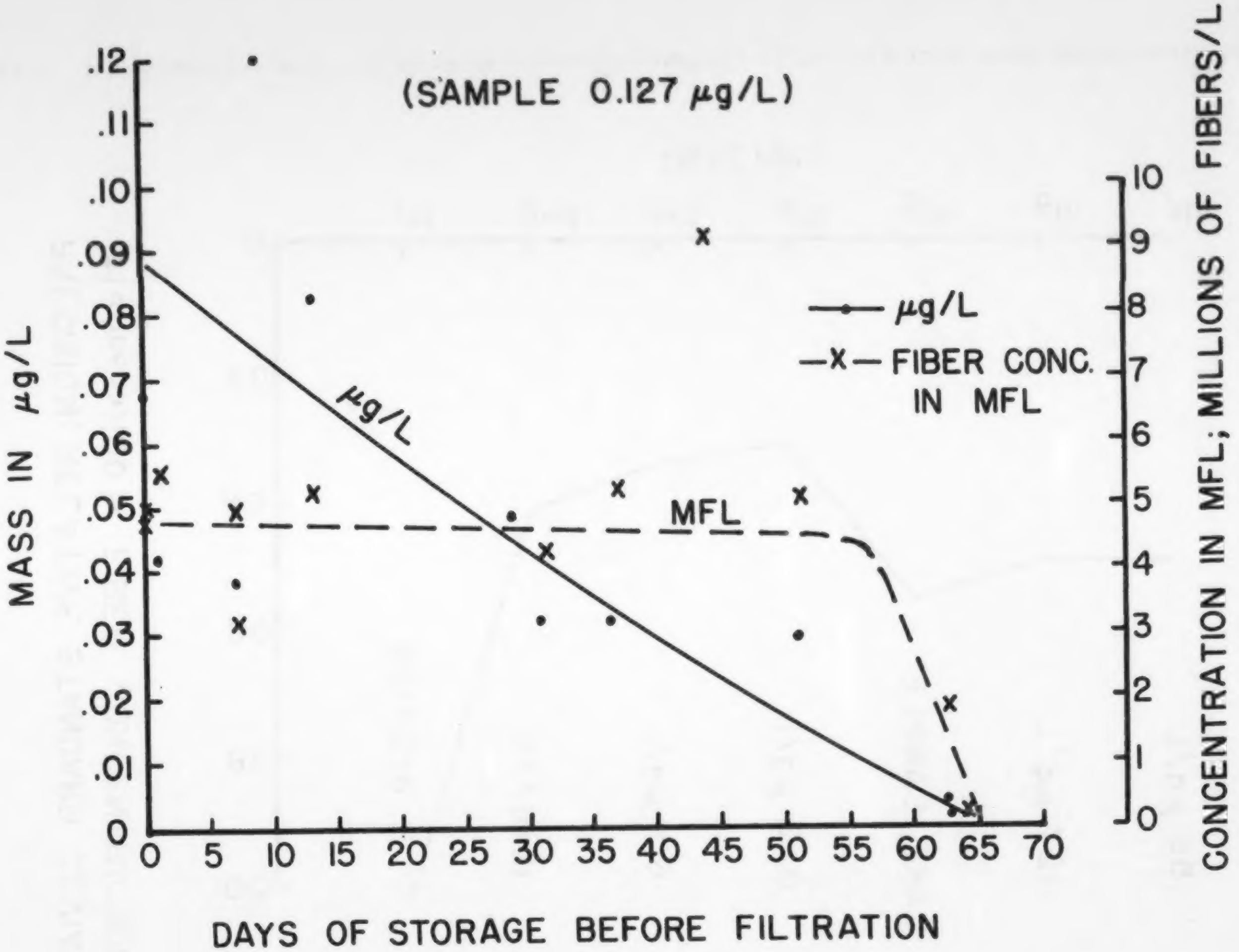

Figure 1. Effect of storage time on measured chrysotile fiber mass and concentration.

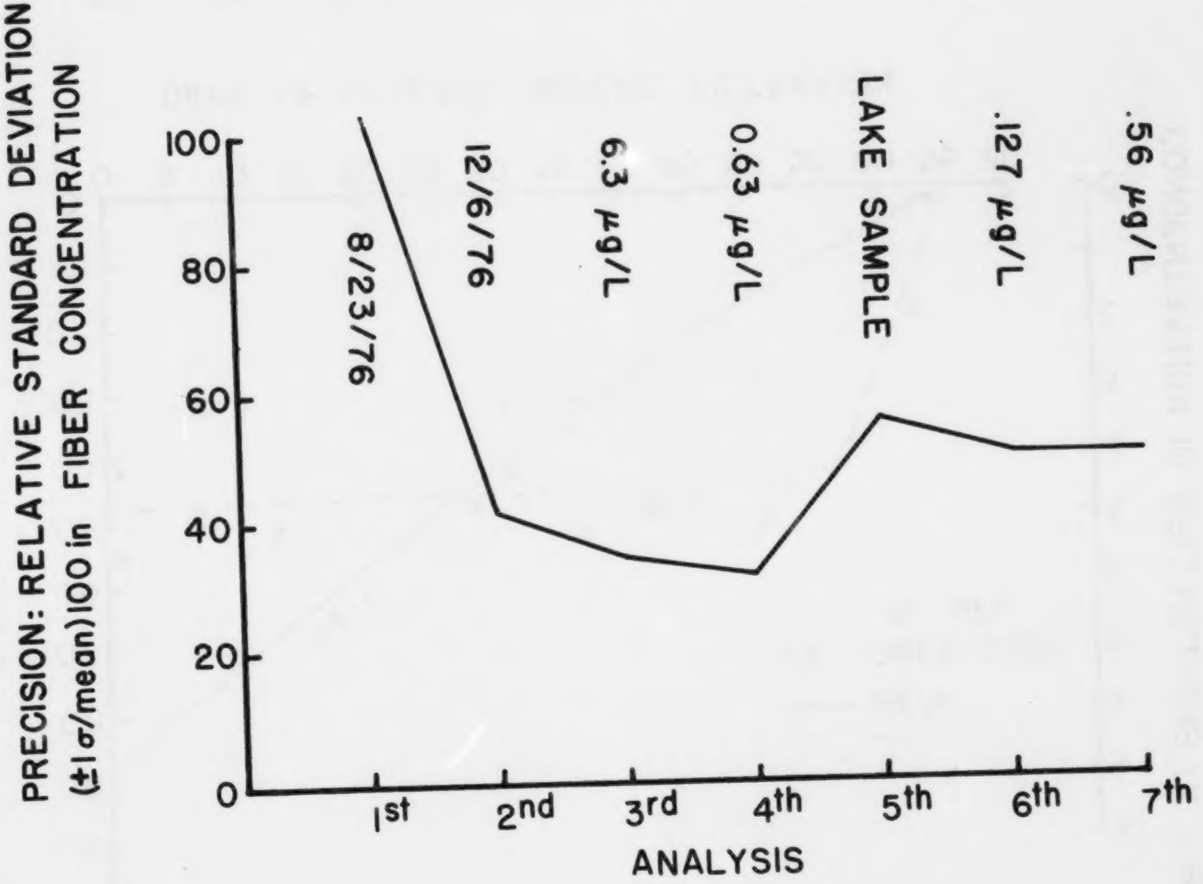

Figure 2. Interlaboratory precision in seven consecutive analysis of the chrysotile fiber concentration in water.

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- D. Benefiel, Dow Chemical-Freeport
- K. Bishop, Minnesota Department of Health
- E. Chatfield, Ontario Research Foundation
- K. S. Chopra (Chairman), Union Carbide Corp.
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- I. Stewart, McCrone Associates
- G. Surrus, Dow Chemical-Sarnia
- A. Szirmae, U. S. Steel
- G. Yamate, Illinois Institute of Technology Research Institute

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[4] C. Melton of Battelle performed the analysis of the leached fibers for the ASTM committee.

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CONSIDERATIONS IN THE ANALYSIS AND DEFINITION OF ASBESTOS USING ELECTRON MICROSCOPY

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Abstract

The most widely used working definition for asbestos in electron microscopy analysis was established on neither a physical nor health related basis. It derived from the definition applied to occupational monitoring using optical microscopy, without regard for the differences between the two types of instruments. The definition has no lower limit on fiber length and an inappropriate lower limit on aspect ratio. This, plus the reporting of data with poor counting statistics, has detracted from the effective use of electron microscopy in quantifying nonoccupational exposures to asbestos. Thus, much of the time and effort expended so far on this type of analysis has no long term value. The purpose of this paper is not to present new data, but rather to summarize some difficulties with current analyses. Further, we propose for consideration some operational definitions and procedures that are more physically meaningful and cost effective than those presently in use.

Introduction

Until recently, optical microscopy has been the only method used for monitoring concentrations of asbestos in the work place. Because of the limited resolution of the optical microscope, the transmission electron microscope (TEM) is now widely used to measure concentrations of sub-microscopic asbestos particles in nonoccupational samples [1][1]. Even though the TEM is a powerful instrument, rigorous measurement of fiber concentration is a difficult problem because of a large number of variables involved in the procedure [2].

This symposium is being held to review and identify those aspects of the analysis that can be quantified and to propose the development of a set of standards. Therefore, this is an ideal forum to address those questions which left unresolved will continue to affect results in spite of attempts to standardize.

This paper is divided into two sections. In the first part, some statistical considerations are reviewed; in the second part, implications of the working definition of asbestos used in electron microscopy are discussed.

1. Analytical Considerations

Asbestos analysis involves several separate phases, from sample collection to data interpretation. The goal is to determine the asbestos concentration from a microscopic count, and so the ultimate result is critically dependent on the validity of the assumptions and procedures used in each phase. The basic assumptions are: (1) representative samples collected; (2) particulates are uniformly deposited on the filter substrate; (3) no contamination introduced during sample preparation and analysis; (4) no instrument or operator influence on the analysis and no misidentification of nonasbestos fibers; (5) collection and analysis of all asbestos fibers in the sample; (6) no effect from sample dilution or repreparation steps.

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[1] Figures in brackets refer to the literature references at the end of this paper.

The first four assumptions have been extensively examined and are now being rigorously studied in the NBS/EPA program (Steel et al., [3]). Since the collection efficiency of any filter is dependent on the size of the particles, the fifth assumption cannot be valid. Further, as will be discussed in the following the sixth assumption can lead to erroneous results if not carefully and systematically validated.

The sixth assumption is required because of constraints on the total mass per unit area that can be analyzed. Normally, the electron microscope analysis of asbestos in air or water samples requires dilution of the original sample whenever the expected loading will exceed 5 to 20 µg/cm² [4]. A small portion of the results of a recent large-scale study of mine water effluents are presented in Table 1. Noted here are calculated fiber concentrations ranging from 10^6 to 10^{11} f/l, based on the detection of only one or two fibers per sample. Examination of the data reveals several inadequacies that have important implications. By dividing the reported concentration by the number of fibers counted, the unit fiber concentration can be back-calculated. The report also indicated that 20 grid openings were analyzed on each sample. Further, it was stated that 100 mL of water were filtered each time. With this information the effective sample dilution and blank levels can be calculated.

Table 1. Electron Micoscope-Determined Concentration of Chrysotile Asbestos in Mine Water Samples.

	Chrysotile*		
Ore	Concentration (f/l)	Mass (gm/l)	Fiber Count
Ag	10^7	10^{-7}	4
Ag	10^{11}	10^{-4}	2
Au	10^9	10^{-6}	1
Cu	10^{11}	10^{-3}	30
Fe	10^{10}	10^{-4}	7
Hg	10^{11}	10^{-4}	11
Mo	10^{10}	10^{-5}	1
Pb/Zn	10^{10}	10^{-5}	4
Ti	10^9	10^{-3}	4
U	10^6	10^{-8}	1
W	10^{11}	10^{-3}	19

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[a] Data extracted from consultant's report to EPA.

Figure 1 shows the calculated concentration in fibers per litre and fibers per gram compared with the calculated dilution factor. It can be seen that the concentration is directly proportional to the dilution and independent of sample type over some six orders of magnitude. Thus, the effective blank level is not significantly different than the calculated concentration for the vast majority of samples.

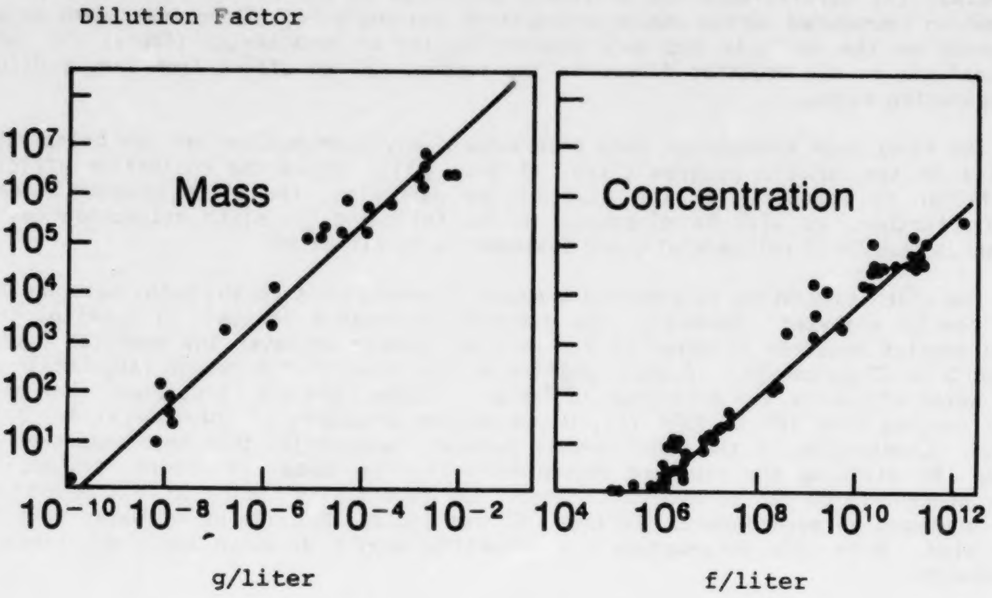

Figure 1. Comparison of calculated dilution factor and reported chrysotile concentrations.

This example analysis illustrates several problems common to all asbestos analysis. There is no uniform method for reporting results, and inadequate regard is given to the effects of sample dilution and the meaning of terms such as "minimum detectable limit" and "effective blank levels". These inadequacies have resulted from the unavailability of a coherent statistical treatment of the data.

To eliminate these deficiencies, we propose several procedures for consideration. Every report of asbestos analysis should include the following:

1. A statement of the population statistics assumed; binomial, negative binomial, Poisson, or other [5].

2. An estimated uncertainty range for the calculated concentration [2]. The uncertainty range associated with the unit fiber concentration and the effective blank level should also be stated.

3. Only those cases where the lower confidence level for the measured concentration exceeds the upper confidence level for the unit fiber concentration or effective blank level (whichever is larger) should be reported as a positive result. For example, assuming the Poisson distribution, the lower confidence level for unit fiber concentration is 0.1 (with $\alpha = 0.05$) and the upper confidence level is 5.6. In order to report a positive result, the analysis would continue until 12 or more fibers were detected. If the analysis is terminated before this point, the results should be reported as indeterminate.

4. In the preparation of samples, the total suspended solids (TSS) for water or total suspended particulates (TSP) for air should be determined and used to estimate the concentration μg/cm² of particulates on the final filter substrate. Those samples that must be diluted in order to achieve the optimal 5 μg/cm² loading should be prepared twice, once at an estimated loading of 10 μg/cm² and another at about 2 μg/cm². Two 200 mesh TEM grids should be prepared for each sample and five openings on each grid analyzed. If the measured concentrations are not statistically equivalent, the heavily loaded sample should be repeated or the result reported as indeterminate. Another advantage of measuring TSP or TSS before the analysis is that, using simple assumptions, a lower limit on the measurable concentration can be estimated as a function of three parameters. The result is a so-called "minimum measurable concentration" for water analysis, as shown in Table 2. The data

shown are based on the following assumptions: (1) 5 $\mu g/cm^2$ loading on the final filter; (2) analysis of 100 fibers in 20 to 200 mesh grid openings ($1.6 \times 10^{-3}\ cm^2$); (3) mean particle mass of 10^{-12} g.

Table 2. Suspended Solid Concentration and Required Sample Aliquot vs. Measurable Asbestos Concentration.

Measurable Concentration (fibers/liter)	Maximum Concentration of Suspended Solids (mg/L)	Sample Aliquot		
10^{2}				
10^{3}	10^{-4}	500	liters (~130 gal.)	
10^{4}	10^{-3}	50	liters	
10^{5}	10^{-2}	5.0	liters	
10^{6}	10^{-1}	500	mL	clean drinking water
10^{7}	1	50	mL	
10^{8}	10	5	mL	river water
10^{9}	10^{2}	.5	mL	
10^{10}	10^{3}	.05	mL	

While these assumptions are not the only considerations in obtaining a good analysis, they do represent a major obstacle when analyzing environmental specimens where the expected absolute fiber count will be small. Even though these recommended procedures may require further development, they do at least provide a starting point for discussion of an otherwise neglected topic.

2. Working Definition of Asbestos

Presently, the most common working definition of asbestos for electron microscope analysis calls for only a 3:1 length-to-width ratio, no minimum length, and the identification of a particle as an amphibole or chrysotile mineral. The shortcomings and ambiguities of such a working definition have been discussed in detail elsewhere [5-8]. It is our intent here to illustrate the effect of this definition on the accuracy of asbestos analysis rather than to argue its inappropriateness alone.

The work of the ASTM Task Force on Asbestos was discussed by Chopra [9], and that of the NBS discussed by Steel et al., [3]. Methods for identifying asbestos were presented by Ring [7] and Yamate [10] at this meeting. From these discussions, it is apparent that techniques are now well developed for mineral identification. Under very carefully controlled conditions, an analysis costing from \$1000 to \$2000 yields a result with a variability from 50 to 100 percent. Even with such very expensive analyses, significant problems remain because of two factors – the aspect ratio and length criteria used in the analysis.

Using the 3:1 aspect ratio fiber definition is responsible for a major fraction of the large analytical cost, mainly because detailed analyses are necessary in order to eliminate the interference by nonasbestos particles. Alternatively, if low-cost analyses are used, large uncertainties are introduced because of misidentification.

The absence of a minimum length in the definition also has several effects:

1. The analyst spends most of this time on the smallest size particles; this results in significant uncertainties in the estimated concentrations of the longer fibers.

2. As shown by Steel et al., [3], under the most carefully controlled conditions, two analysts may arrive at the same average count by counting different objects.

3. Steel et al., [3] also report that a significant fraction of fibers shorter than 2 μm are missed in the analysis. Thus, even though we strive to produce an absolute fiber count, the count is only a fraction of the total concentration and that fraction is clearly dependent on the instrument and operator employed.

4. The reported concentration is not a measure of a known health hazard. Instead, it is a mixed index which includes the concentration of both known and potential hazards. This ultimately limits our ability to identify the individual significance of either group.

The problems listed here are not strictly analytical but rather derive from the definition of asbestos. What is required for a meaningful analysis of fiber concentration is an operational definition which includes bounds on the aspect ratio, length, and width. Clearly, a given analysis will measure only a portion of a distribution. If desired, it is possible to calculate the total concentration from the measurement of a subset of a distribution if the form of the distribution is known. Problems in asbestos analysis arise because subset concentrations are reported implicitly as total concentrations and are compared with results from other laboratories which measured different subsets.

As the basis for the formulation of a meaningful working definition of asbestos, we propose the following:

1. For a routine method, a minimum aspect ratio of 10:1 should be used in a screening analysis or survey. Existing data indicate that this would not affect the chrysotile analysis at all and amphibole analysis only when the sample contains a significant percentage of acicular nonasbestos particles [11-16]. While this would undoubtedly result in missing 5 to 20 percent of the short asbestos particles, it would eliminate 70 to 80 percent of the nonasbestos particles from consideration.

2. A lower length limit for routine electron microscope analysis should be adopted. On the basis of available information, a reasonable limit would be somewhere between 0.75 and 2.0 microns [3].

3. Asbestos analyses should be grouped into at least three size fractions and acceptable uncertainty levels defined for each range. For example, the length categories less than 2, 2 to 5, and greater than 5 μm might be chosen, and a 50 percent relative error defined as the minimum level of acceptance for each size range.

Summary

Analysts need to examine the objectives in performing analyses for asbestos. Is it to provide an absolute measure of the concentration, or is it to produce a reliable, reproducible measure or index which allows comparison of exposures?

In the past we have attempted to obtain answers to two questions simultaneously. First, a research-type question, "What is the significance of small, low-aspect ratio particles?" Second, "What are the concentrations of known hazards in environmental samples, and by what procedures can they be measured?" Electron microscope analysts must treat these as separate questions.

Analysts have been waiting for the medical community to define the size and shapes of significance. Unfortunately, answers to the above questions may not be forthcoming if analysts cannot provide a meaningful data base.

Now, at this session on standardization, it is time to define the statistical requirements and analytically based working definition of asbestos that will permit reliable and effective analyses by electron microscopy.

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COMMENTS ON THE ACHIEVABILITY OF A VALID ASBESTOS STANDARD FOR TEM COUNTING

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1. Introduction

The validity of any standard reference material is only as good as the validity of the sample preparation and analytical methodology used to characterize the standard. In the case of standards for the determination of fiber counts by electron microscopy, the literature is somewhat vague on the validity and the reproducibility of specimen counting procedures. Most electron microscopists, when questioned on the subject, would generally agree that results are valid within an order of magnitude but to a certain extent this has been a gut-feeling or an inspired guess rather than the results of any tests performed to confirm this. Such round-robin tests as have been performed have generally been confounded statistically by a confusing medley of comparisons between sample preparation methodologies, different instrumentation used for the analyses, and variability in operator experience both on these instruments and of asbestos identification. It is not the object of this paper to further cloud the issue but, instead, to provide some gleam of hope that standards may, indeed, be valid.

1.1 Background

The work described in this report was performed in late 1974 and early 1975 with the object of establishing whether or not a particular sample methodology would give accurate and reproducible results for the determination of asbestos in water samples.

Walter C. McCrone Associates, Inc. had been contracted by the EPA to perform a sampling of the impact of point and non-point sources of asbestos on waterborne levels of asbestos. This program was to be conducted nationwide, sampling and filtration were to be carried out on-site and the filters were to be returned to our Chicago laboratory for subsequent analysis. The filter medium chosen for these samples was the Millipore® 0.45 µm pore size, 47 mm diameter mixed ester filter and samples were collected by vacuum filtration using the standard Millipore glass funnel and flask arrangement. Sample preparation was to be by the condensation washing method in a Soxhlet condenser. Without discussing in detail the pros and cons of Millipore vs. Nuclepore®, condensation washing vs. Jaffé Wick, the rationale for the selection of this procedure can be briefly summarized as follows:

1. Sampling was to be performed in a mobile laboratory and samples were to be retained in the laboratory until its return to home base. In many instances this meant three to four weeks of travel on the road in the laboratory. Although there was no hard data at that time[1] to support this assumption, it was felt by all the investigators concerned that Millipore filters would have a better fiber retention than the smoother Nuclepore filters under such circumstances, bearing in mind that facilities to carbon coat the Nuclepore filters in the field were non-existent.

2. Previous work carried out independently by McCrone Associates and by Jack Murchio of the University of California, Berkeley, had indicated that, in the case of glass fibers at least, there was a tendency for large fibers to be lost during Jaffé Wick preparation of Nuclepore filters. As most of the samples on the contract were to be taken close to point sources, it was felt that there was a real danger of the loss of large fibers if Nuclepore filters and the Jaffé Wick techniques were used.

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[1] A limited sample performed at a later date tended to confirm this finding (Stewart, I. M., Proc. F.D.A. Office of Science Symp. on Electron Microscopy of Microfibers, 1976, p. 96 and Table II.

3. Comparative studies performed by the EPA during the Reserve Mining case in which samples had been split between McCrone Associates and Jack Murchio, the latter using a Jaffé Wick technique, had shown good comparability of fiber counts between the Millipore condensation washing technique used by McCrone Associates and the Jaffé Wick as used by Murchio[2].

Taking these criteria into consideration therefore, the decision was made to use Millipore filters and the condensation washing technique.

The series of tests described in this paper were, therefore, devised to establish some of the parameters which might affect the reproducibility and accuracy of the analysis of Millipore filters, condensation washed.

Three test series were conducted:

1. An extensive examination of samples taken from one individual filter designed to test the reproducibility of a single operator, the variation between different operators and the evenness of distribution on the filter.

2. A series of tests aimed at testing the reproducibility between different filters produced from different aliquots of the same sample.

3. A series of dilution tests aimed at assessing the accuracy of the count. The tests are described in the following section.

1.2 Materials and Methods for Conducting Tests

In all groups of tests considered, the sample preparation method was identical and was a direct transfer method using a Soxhlet extractor with acetone as a solvent. All the sample measurements were made on a JEOL JEM-200 transmission electron microscope operating at 200 kV. A standard suspension was prepared of chrysotile asbestos from the Jeffrey Mine in particle-free water. We had worked extensively with this suspension in connection with a sub-contract from an FDA contract on animal studies and, therefore, felt that we knew the suspension quite well. The suspension was prepared in water that had been filtered three times, the last filtration being through a 0.2 μm pore size membrane filter. Three filters were prepared in succession from this suspension; the remaining unfiltered suspension was then diluted by a factor of 10 and a fourth filter prepared, then by a further factor of 10 and a fifth filter prepared. These five filters formed the basis for the three test series. Measurements made on these filters are listed in Table 1.

1.3 Test Series I

The first of the five filters was the subject of a detailed statistical analysis (performed by Dr. Neill of the Illinois State Water Survey), the object of which was to determine the variation in particle distribution on the filter. Thirteen test locations were established on the filter as shown in figure 1. At each of these 13 test locations an electron microscope grid was prepared and examined. The sample was prepared on finder's grids which enable the precise re-location of the area examined on the grid. In this way it was possible for more than one observer to measure the asbestos content on identical areas. Three grid squares on each grid were examined by the first observer to give a total of 39 observations from the one filter. Each grid square on the finder's grid is equivalent in area to approximately three grid squares on a regular 200-mesh TEM grid.

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[2] A preliminary report on asbestos in Duluth, Minnesota area, Office of Technical Analyses, Office of Enforcement and General Counsel, U.S. EPA, January 1974, Appendix III.

Table 1. Fiber Counts on Standard Chrysotile Suspension.

Filter	Grid	Location	Observer 1	Observer 2
1A	1	DELTA 1	0.31×10^8	
	1	X2	0.37×10^8	
	1	M-0	0.32×10^8	
	2	E1	0.32×10^8	
	2	Y2	0.36×10^8	
	2	DELTA 1	0.33×10^8	
	3	1-2	0.38×10^8	
	3	F-1	0.31×10^8	
	3	R1	0.32×10^8	
	4	C-0	0.30×10^8	
	4	A2	0.31×10^8	
	4	6-3	0.28×10^8	
	5	X3	0.34×10^8	
	5	H2	0.34×10^8	
	5	R0	0.26×10^8	
	6	G3	0.25×10^8	0.25×10^8
	6	2-1	0.37×10^8	0.38×10^8
	6	7-2	0.35×10^8	0.44×10^8
	7	X-3	0.28×10^8	
	7	P-3	0.32×10^8	0.30×10^8
	7	K1	0.31×10^8	
	8	DELTA 2	0.35×10^8	
	8	1-2	0.39×10^8	0.37×10^8
	8	D-3	0.36×10^8	
	9	M-1	0.39×10^8	
	9	7-0	0.36×10^8	
	9	4-1	0.37×10^8	
	10	1-1	0.33×10^8	0.35×10^8
	10	H-2	0.35×10^8	
	10	0-2	0.32×10^8	0.34×10^8
	11	L-0	0.34×10^8	
	11	5-1	0.33×10^8	0.24×10^8
	11	W-2	0.37×10^8	0.42×10^8
	12	K-0	0.35×10^8	
	12	S-0	0.35×10^8	
	12	6-1	0.35×10^8	

Table 1 continued.

Filter	Grid	Location	Observer 1	Observer 2
1A	13	Q-0	0.31 x 10^8	
	13	T-1	0.34 x 10^8	
	13	Y-1	0.36 x 10^8	
1B	01	DELTA 2	0.23 x 10^8	
	01	7-1	0.27 x 10^8	
	01	G3	0.22 x 10^8	
	02	E3	0.25 x 10^8	
	02	S1	0.27 x 10^8	
	02	5-1	0.27 x 10^8	
	03	N1	0.30 x 10^8	
	03	DELTA 0	0.22 x 10^8	
	03	Q0	0.26 x 10^8	
	04	L-0	0.23 x 10^8	
	04	1-1	0.27 x 10^8	
	04	R-1	0.24 x 10^8	
	05	4-0	0.28 x 10^8	
	05	F3	0.27 x 10^8	
	05	R-0	0.28 x 10^8	
1C	01	A3	0.32 x 10^8	
	01	DELTA 1	0.29 x 10^8	
	01	6-0	0.26 x 10^8	
	02	T1	0.29 x 10^8	
	02	W-0	0.26 x 10^8	
	02	L-1	0.27 x 10^8	
	03	F-2	0.26 x 10^8	
	03	F1	0.26 x 10^8	
	03	4-0	0.27 x 10^8	
	04	N-1	0.27 x 10^8	
	04	6-1	0.27 x 10^8	
	04	Q-0	0.24 x 10^8	
	05	J3	0.30 x 10^8	
	05	H2	0.28 x 10^8	
	05	DELTA 2	0.29 x 108	

Table 1 continued.

Filter	Grid	Location	Observer 1	Observer 2
2	01		0.23×10^7	
	02		0.25×10^7	
	03		0.20×10^7	
	04		0.28×10^7	
	05		0.23×10^7	
3	01		0.31×10^6	
	02			
	03		0.24×10^6	
	04		0.70×10^6	
	05		0.35×10^6	

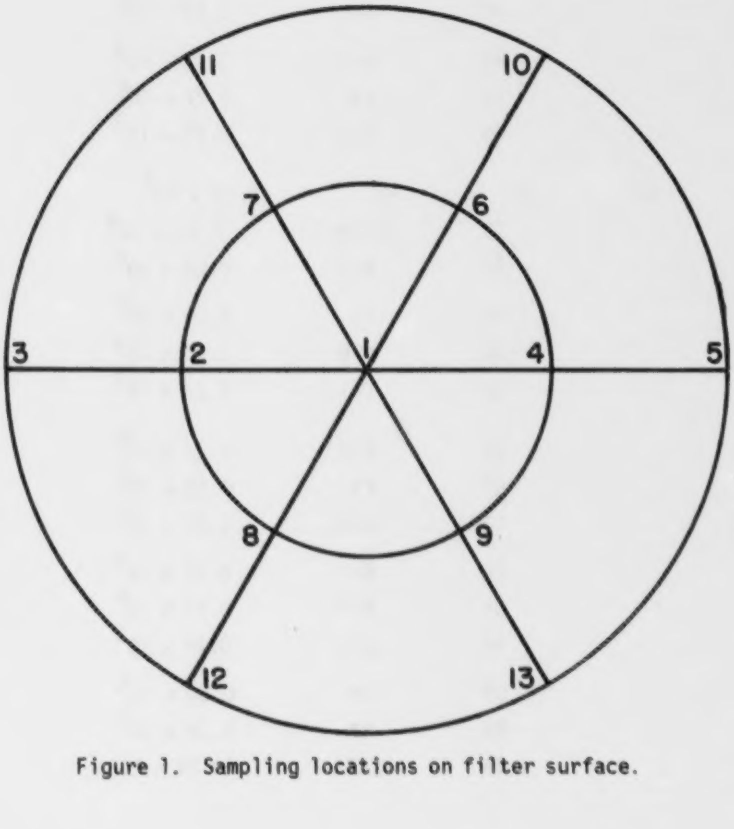

Figure 1. Sampling locations on filter surface.

Summarizing the data from Table 1 in Tables 2 and 3, it can be stated that good reproducibility was obtained from different locations on the same filter indicating a uniformity of distribution which, in fact, exceeded our expectations, bearing in mind that the approximate 10 percent standard deviation in fiber counts for Observer 1 represents both the variation of the observer's interpretation of what constituted a fiber plus the variation of distribution on the grids and filter. Additionally, the statistical treatment of the length and width distributions by Dr. Neill showed no systematic variations in these parameters which would have been indicative of size segregation effects on the filter.

Table 2. Statistical Data Derived from Table 1 for Filter 1.

Filter	Grid	Mean Fibers/Liter Observer 1	Observer 2
		Three readings per location	(No. of readings in parenthesis)
1	1	0.333×10^{8}	
	2	0.337×10^{8}	
	3	0.337×10^{8}	
	4	0.297×10^{8}	
	5	0.313×10^{8}	
	6	0.323×10^{8}	0.357×10^{8} (3)
	7	0.303×10^{8}	0.30×10^{8} (1)
	8	0.367×10^{8}	0.37×10^{8} (1)
	9	0.373×10^{8}	
	10	0.333×10^{8}	0.345×10^{8} (2)
	11	0.347×10^{8}	0.33×10^{8} (2)
	12	0.35×10^{8}	
	13	0.34×10^{8}	

	Mean Value	Std. Deviation	No/Readings
Observer 1	0.335×10^{8}	9.88%	39
Observer 2	0.3433×10^{8}	19.08%	9

Table 3.

Run No.	No. of grid Squares Examined	Fibers/Liter	Rel. S.D.
1a	39[a]	33.6×10^6	9.75%
1b	15[b]	25.7×10^6	8.96%
1c	15[b]	27.4×10^6	6.85%
2	20[c]	2.38×10^6	11.09%
3	30[d]	0.36×10^6	48.62%

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[a] Three grid squares on each of 13 different grids prepared from the same filter, freshly prepared ultrasonerated suspension.

[b] Three grid squares on each of five different grids prepared from the same suspension as 1a, allowed to settle then manually shaken to redisperse. 1b and 1c represent two separate filters.

[c] Four grid squares on each of five different grids prepared from suspension of 1a, b, and c diluted to 1/10th concentration.

[d] Six grid squares on each of five different grids prepared from suspension of 1a, b, and c diluted to 1/100th concentration.

When a second observer measured areas which had previously been observed by the first one we again get good reproducibility in terms of the average fiber count, although in this instance the standard deviation has in fact risen to approximately 20 percent.

Fiber levels measured by Observer 1 on the second and third filters prepared from the same standard chrysotile suspension did not differ significantly from each other but did show a significant decrease in fiber count compared to the first filtration. Though both filters did show decreases in length, width, and mass, the decrease could not be regarded as statistically significant, it was therefore impossible to determine whether the decrease in fiber count was due to failure to re-suspend all the large settled particles, to entrapment of smaller particles on the container walls, or whether both mechanisms might have been operative.

On examining filters representing dilutions from the original standard suspension, Table 3, there was indeed an order of magnitude decrease in fiber count observed between the successive filtrations. One should note that when the data for the 100-fold dilution is considered the standard deviation has increased markedly, reflecting now the effect of probability statistics in terms of whether or not a fiber would be observed in a particular grid square (at the 100 dilution level the fiber count should have averaged one to two fibers per finder's grid square).

2. Summary

To summarize the results presented in this paper then, it seems that there is good hope that a true, accurate, standard can be produced and can be made reproducible for the analysis of asbestos by TEM, provided we ensure a sufficiently high loading for statistically valid counts to be made from it. This work suggests that a minimum loading of the order of 10 fibers per 1000 square micrometers of filter is desirable.

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MEMBRANE FILTER METHOD: STATISTICAL CONSIDERATIONS

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Abstract

The historical evolution of the understanding of errors associated with workplace sampling for airborne asbestos using the membrane filter method is presented. Statistical considerations of the membrane filter method are illustrated using analytical and empirical results. Possible sources of error and those which should be considered are discussed. The importance of the form of the distribution used to quantify the reliability of the method is shown using theoretical and empirical findings. In particular, it is shown that Gaussian assumptions can be very misleading when the error distributions are skewed. Finally, the characterization of the reliability of the method is discussed.

Most of the early work in measuring workplace asbestos levels was carried out using techniques which yielded results in the form of total particulate concentrations. The membrane filter method, which yields results in the form of the number of fibers of specified dimensions, was pioneered by a British textile company in the 1960's. However, the widespread use of the membrane filter method did not occur until the late 1960's and early 1970's.

The evolution of workplace monitoring of asbestos has been the source of a number of interesting theoretical and analytical statistical problems, some of which are still under investigation.

Some studies have investigated the relationship between different monitoring techniques. One of those investigations, reported by Ayer, Lynch, and Fanney (1965) [1][1], used a study design involving side-by-side samples of the membrane filter and impinger (which yield particle counts) methods. Investigations of the relationship between the two methods in the mining and milling industry has been carried out by Gibbs and LaChance (1974) [2] and Dagbert (1976) [3]. The results of those studies did not support the use of a single conversion factor. It is predictable that there is interest in the relationship between any two techniques that are used to measure asbestos levels. However, the estimation methodology for a conversion factor between two observations, both of which are subject to error, must take those errors into account. Thus, a classical least squares regression line is not suitable.

Any attempt at a characterization of the membrane filter method is complicated by the fact that the technique known as the membrane filter method has differed between countries and, even within countries, has actually changed over the years. Part of the explanation is that sampling and analytical equipment and methodology have undergone change. The effect has been substantial. For example, it was estimated by Steel (1979) [4] that a British workplace measurement of 2 f/cm^3 in the 1960's would likely have been on the order of 10 f/cm^3 if it had been measured using today's membrane filter sampling and analytical techniques.

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[1] Figures in brackets indicate the literature references at the end of this paper.

To assess the uncertainty of a workplace measurement for a single day, it is necessary to characterize the errors inherent in the entire sampling/analytic procedure. However, when evaluating the uncertainty of an assessment of longer term (i.e., over days, months, or years) exposure, temporal and spatial variation of the dust cloud must be considered in addition to the error inherent in the sampling/analytic procedures used for a single day. Leidel, Busch, and Lynch (1977) [5] made some thoughtful contributions on these considerations in the NIOSH (1977) [5] publication on sampling strategies.

The remainder of this discussion will deal just with issues concerning the characterization of the variability of the membrane filter method for a determination on a given day using only one sample. Much of the investigation of the inter- and intralaboratory reliability of the method has focused on the analytical equipment and methodology rather than the entire sampling/analytic procedure. Considerable attention has been given to whether or not a Poisson distribution adequately describes the distribution of fibers on a filter. Both intra-wedge and inter-wedge variability have been investigated. An exhaustive study of three filters by six counters was reported by Conway and Holland (1973) [6] from a study for the asbestos industry. That study found more inter-wedge variation on a single filter than would be predicted by the Poisson distribution. Other studies, such as an intercounter-intermicroscope-intrafilter study by Leidel and Busch (1974) [7] and an intralaboratory-intrafilter-intracounter study by Lynch, Kronoveter, and Leidel (1968) [8] clearly support the hypothesis that empirical variability is much greater than would be predicted by the Poisson distribution alone. It should be emphasized that the appropriateness of a Poisson assumption for intrafilter variation is not of major importance in the "bottom-line" assessment of uncertainty. To characterize the inherent variability of the complete process, factors such as intercounter variability and sampling error must be added to the intrafilter variation. In addition, different laboratories, equipment, and methods can have a pronounced effect.

In 1976 some of the theoretical problems of the counting procedure were explored and it was found that the NIOSH counting rules produced a bias on the high side (Cooper et al., (1978) [9]). That is, the longer the fiber, the more likely it was to be counted. That type of bias has implications when fiber size distributions are characterized, although it may be negligible in many situations. Another interesting result that was found by analytical investigation is the theoretical lower bound on the variation if the distribution of fibers is actually uniform. By changing the counting rule, the counting error was actually reduced. In other words, the theoretical lower bound based on the Poisson distribution is actually lower than 1/E(N), where N is the total number of fibers counted. There is also a very small bias due to the stopping rule (Johns-Manville (1976) [10]).

The bottom line for all of these investigations is what sort of uncertainty should be given to empirical results? It is known that a repeat measurement by the same counter using the same slide and equipment is subject to error. It is also known that another counter using the same materials and equipment will introduce more variability than a single counter. If another laboratory is brought into the picture, additional uncertainty is introduced. Additional variability can be attributed to sample replication. There is a miriad of combinations of variables which can be investigated for attributable variability.

The precision of the method has been characterized using the coefficient of variation. The coefficient of variation has generally been given as a function of the expected total number of fibers counted. Even through the Poisson does not describe total variation, the number of fibers counted is still an important factor. Therefore, it is resonable to take the total number of fibers into account in order to estimate coefficients of variation.

The estimation of the coefficient of variation can be very difficult, since large samples from the distribution of interest are often not available or even possible with environmental samples. If many observations are available, they often are taken only two or three at a time. The number of simultaneous personal samples which can be taken on a single worker has obvious limitations. Thus, the statistical comfort that statisticians derive from large samples is simply not available.

The underlying distribution can be important in many applications of statistics. The underlying distribution is crucial when the variability is expressed as the coefficient of variation. Normal (or Gaussian) assumptions can be very misleading when the true distribution is skewed to the high values. Nonsymmetrical distributions are likely to be encountered, especially when they are non-negative and "near zero". In other words, if the true value is near zero, and negative values are known to be impossible, large negative errors are ruled out. However, large positive errors are not ruled out.

To illustrate, consider a symmetrical Normal distribution with a mean of 0.2 and a coefficient of variation of 0.4, and a lognormal (i.e., a positively skewed distribution) with a mean of 0.2 and a coefficient of variation of 0.6. These two distributions are quite different. For example, the middle 95 percent interval for the Normal is from 0.12 to 0.28 (i.e., only 5% of the values will be observed outside this range). The corresponding interval for the lognormal distribution runs from 0.06 to 0.51.

What about sample coefficients of variation from such distributions? Since it is often difficult to obtain more than two replicate samples, consider samples of size two. A Monte Carlo study of 1000 independent sample coefficients of variation from these two distributions was undertaken and the results are given in figure 1. The sample coefficients of variation from the two distributions are similar. In other words, it is difficult to distinguish between the two distributions by looking at the sample coefficients of variation since the range and likelihood of the possible sample coefficients are similar. Of course, this does not prove anything, it is intended only to illustrate that estimation schemes based on Gaussian distributional assumptions are not appropriate.

In late 1975, early 1976, J-M conducted an in-house simultaneous sampling replicate counting study (Johns-Manville (1976) [10]). Ten workplace locations were selected to give a variety of airborne asbestos fiber concentrations and other contaminants. A variety of sampling times were also selected. Six simultaneous samples were taken at every sampling location for each sample time period. The filters were fixed in a generally circular pattern and separated by 4 to 6 inches. One hundred ninety-two filters were collected.

Those data were submitted to OSHA as a part of comments with respect to an October, 1975 proposed rulemaking (Johns-Manville (1976) [10]). The information submitted eventually led to a change in the counting rules in the NIOSH procedure. Also, NIOSH used those data to estimate a coefficient of variation curve, expressing the CV as a function of the total fibers counted in 100 fields. Figure 2 shows the resulting CV curve from Leidel et al., (1979) [11].

There are two points that should be made regarding the estimates:

1. Only within-filter and within-laboratory replicates were used.

2. It was assumed that the underlying distribution of errors was normal.

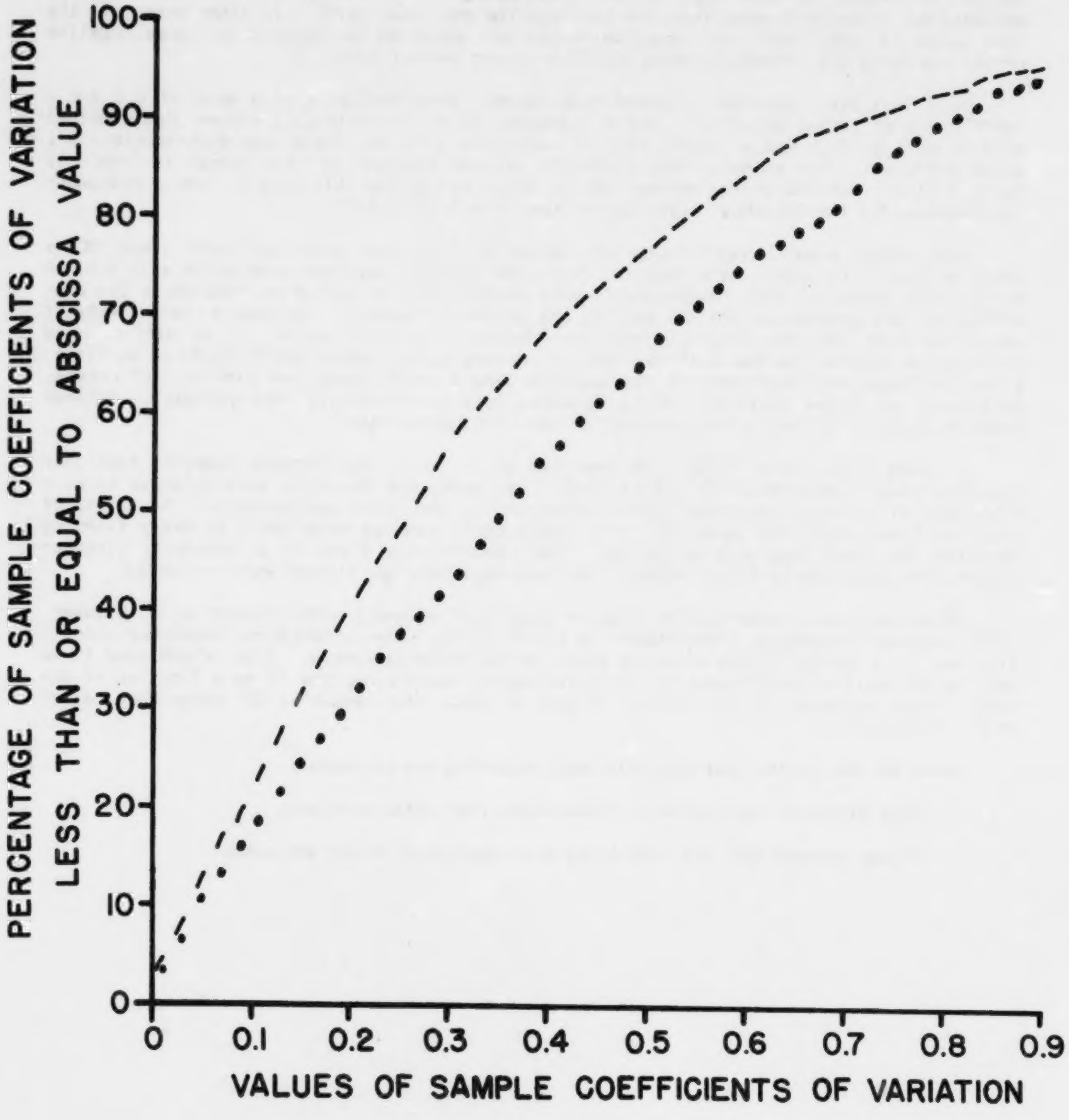

Figure 1. Cumulative distributions of sample coefficients of variation for samples of size two from a Monte Carlo study:

1,000 Repetitions

Lognormal Distribution Mean = 0.2 •
CV = 0.6

Normal Distribution Mean = 0.2 –
CV = 0.4

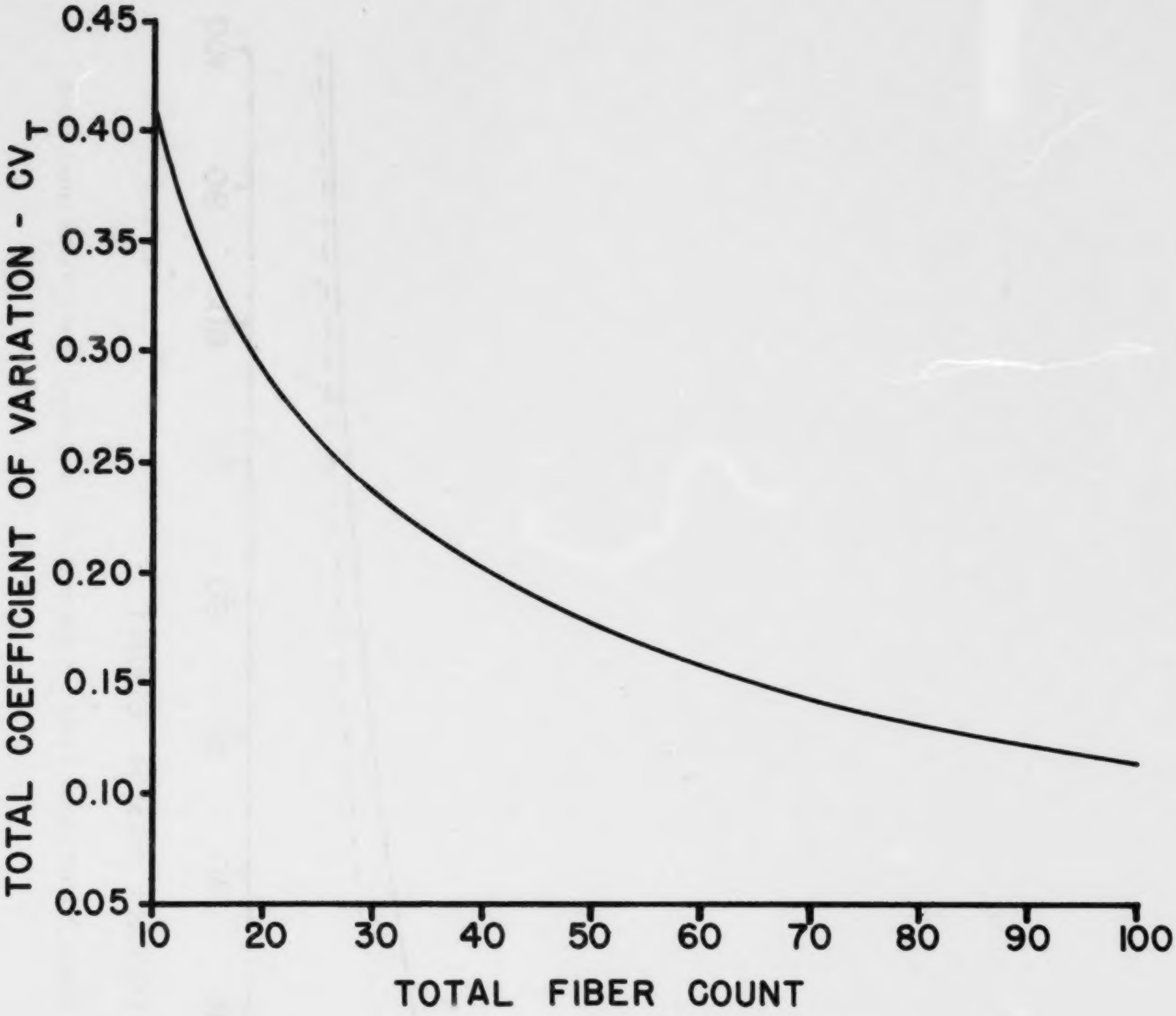

Figure 2. Total coefficient of variation as a function of total fiber count (including pump error). This figure appears as figure 3 on page 81 of Leidel et al., (1979).

The curve published and currently used by NIOSH, and the theoretical minimum based just on Poisson error in counting are shown in figure 3 with a scale change. Analysis of those same data using a technique which does not rely on a normal assumption is shown in figure 4, along with the NIOSH curve. (If X and Y are a pair of observations and S^2 is the sample variance for X and Y, then the coefficient of variation is estimated by the square root of $\Sigma S^2/\Sigma XY$, where the summation is over all possible pairs.)

The data which were not utilized in the NIOSH analysis, between laboratories, are shown on figure 5. It is apparent that the variability between laboratories and filters is substantially greater than the variability within laboratories and filters.

The curves are, of course, estimates and subject to uncertainty. They have not been smoothed out, the individual points are presented here. The CV curves have been shown and calculated as associated with the total number of fibers counted. That point was discussed earlier. It is also known that there are other factors.

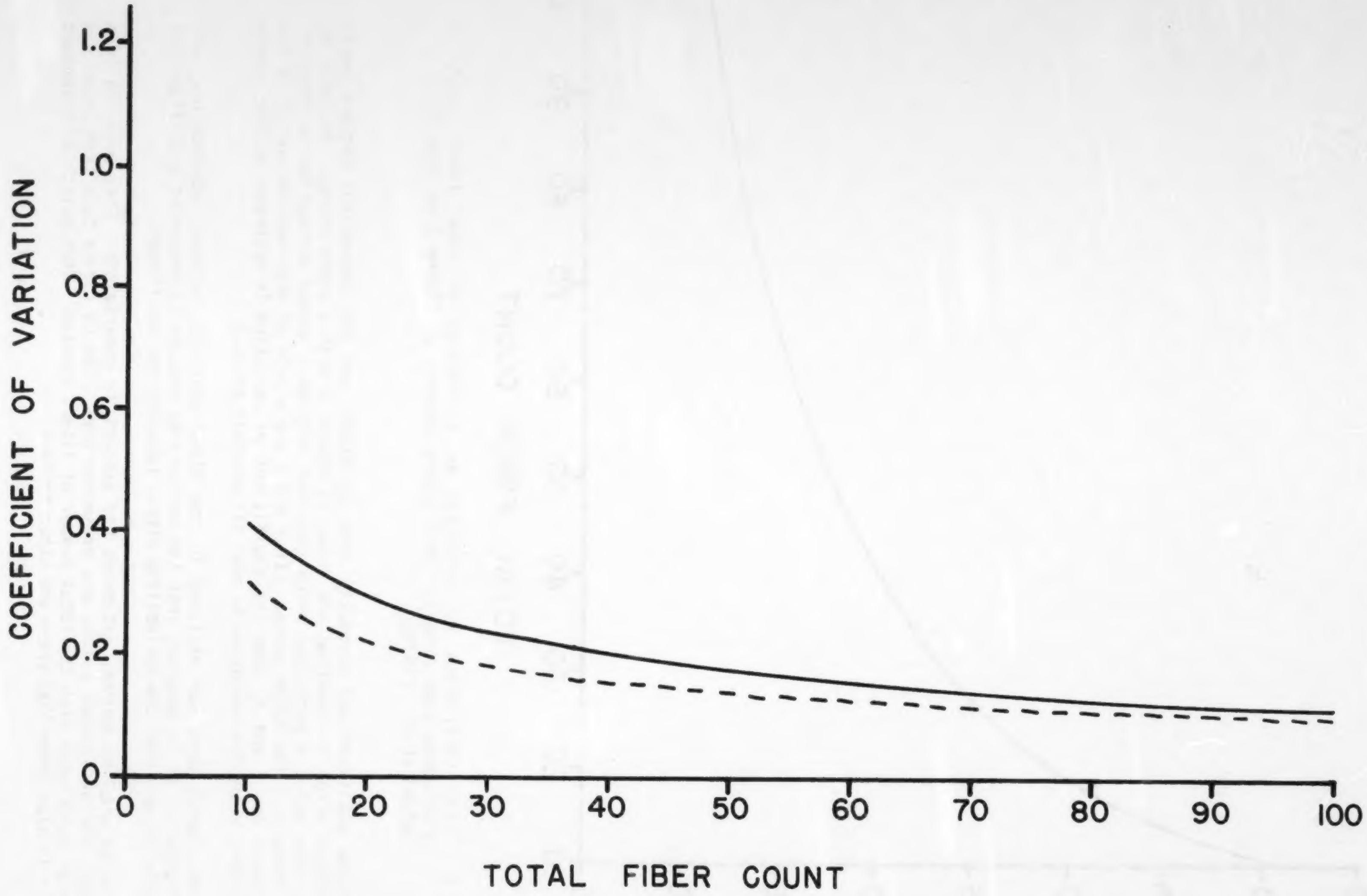

Figure 3. Coefficient of variation from figure 2 (continuous line) and theoretical lower bound from Poisson point count (dashed line).

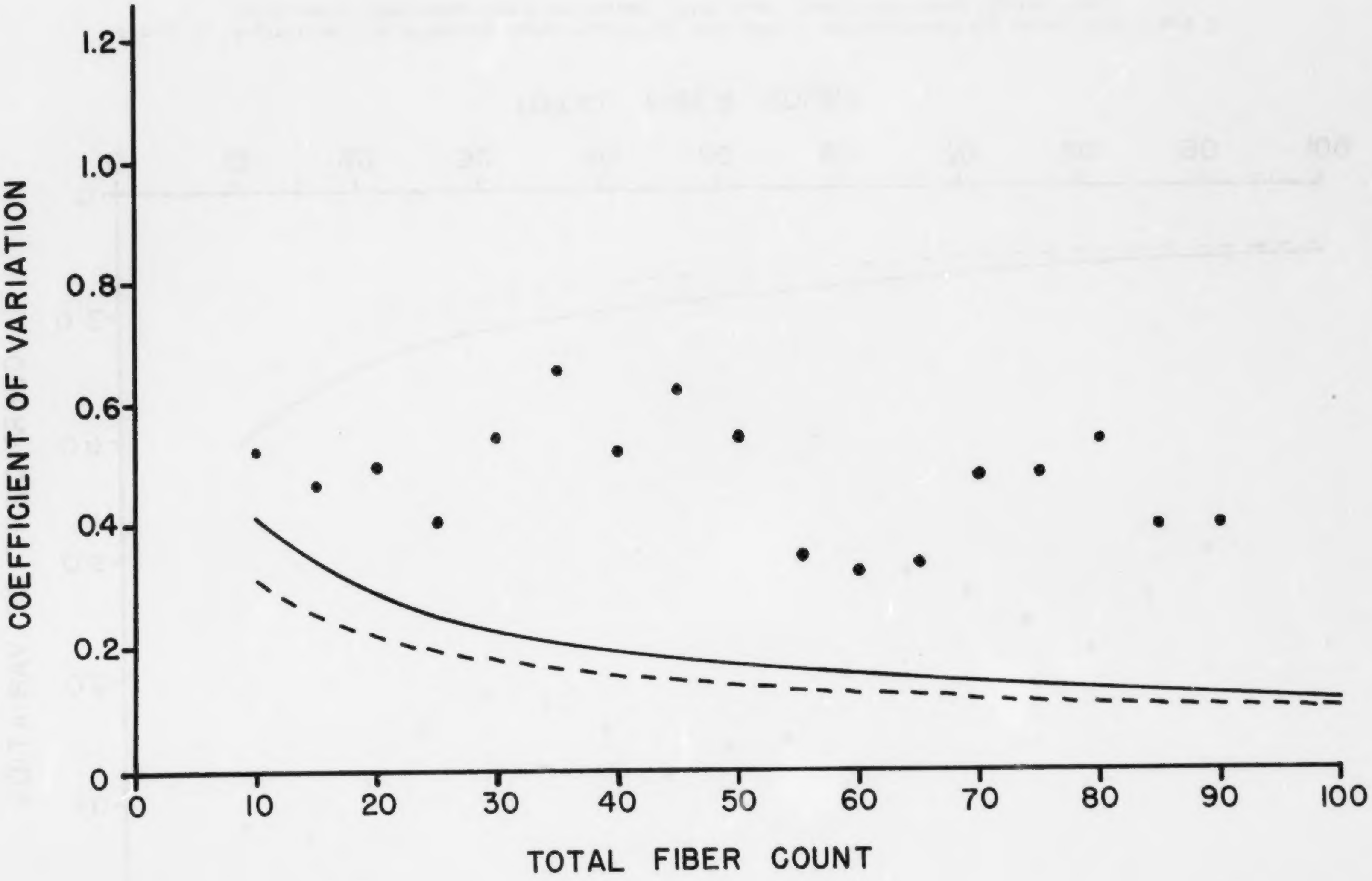

Figure 4. Reanalysis of J-M intralaboratory-intrafilter data (dots – not smoothed) and curves from figure 3: NIOSH curve (continuous line) and Poisson point count theoretical bound (dashed line).

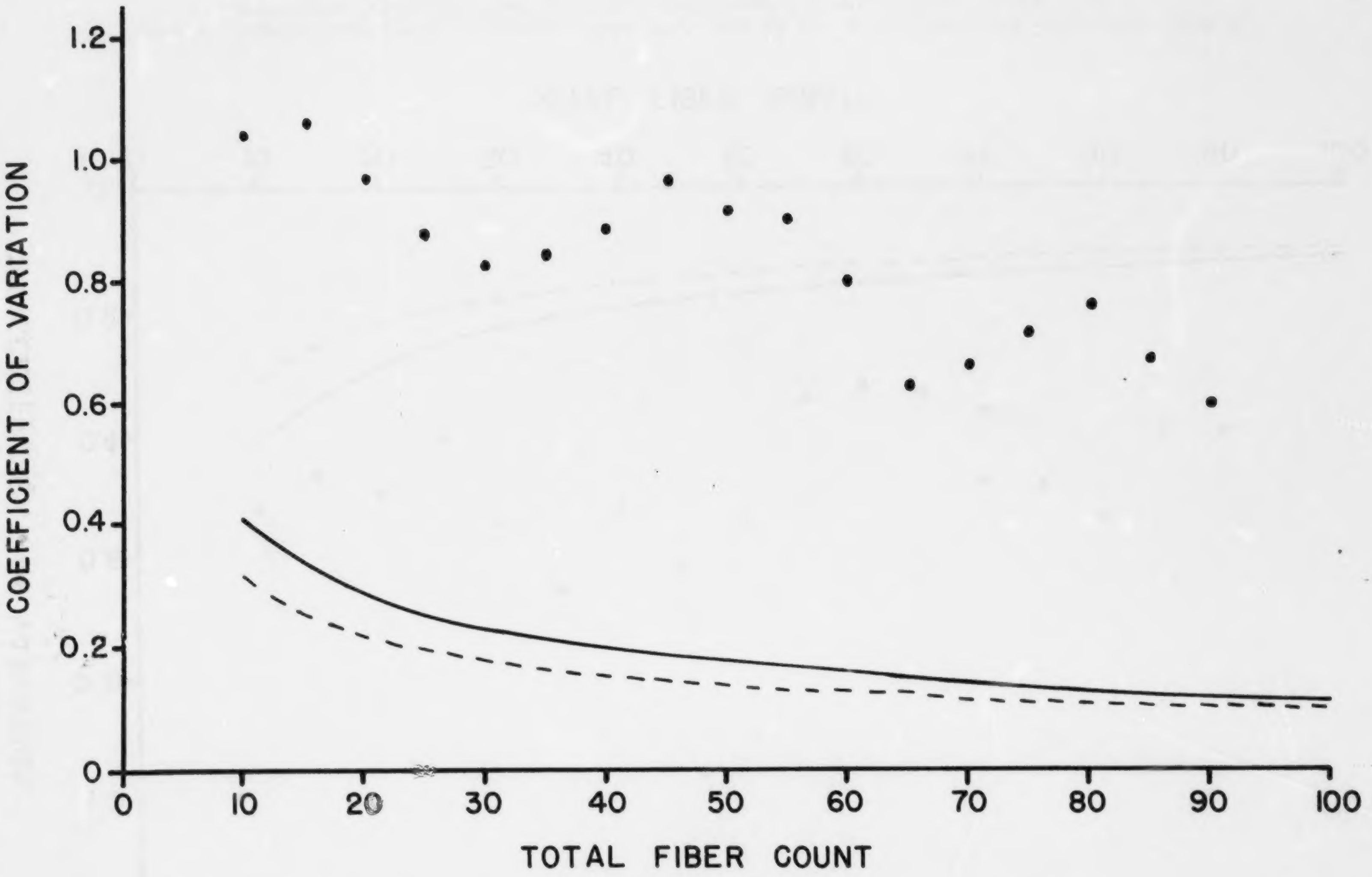

Figure 5. Analysis of J-M interlaboratory-interfilter data (dots — not smoothed) and curves from figure 3: NIOSH curve (continuous line) and Poisson point count theoretical bound (dashed line).

This brief discussion of some of the statistical aspects of workplace asbestos measurements has emphasized one aspect; the importance of methodology which is not sensitive to assumptions which cannot be verified. Other important statistical considerations, which necessarily follow those considered here, such as reliable levels of detection and lowest levels of reliable measurement, have not been discussed.

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SIMULATION OF THE EPA PROVISIONAL METHOD FOR AIRBORNE ASBESTOS CONCENTRATIONS

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Abstract

The most expensive segment of the EPA provisional method for measuring asbestos in the ambient air and the segment that has the greatest potential for error is transmission electron microscopy. Consequently transmission electron microscope analysis is simulated. A hypothetical filter is loaded with fibers. The fiber lengths and widths are generated as a bivariate distribution. The location on the filter and orientation of the filter are generated as uniform random variables. The EPA provisional method is then followed to estimate fiber counts, mass, and size distribution. A plug is taken from the filter, a grid is defined, the grid openings are sampled according to the method. The simulation is presented graphically. Assumptions used in the simulation and the random and systematic sampling errors are discussed.

1. Introduction

The unique physical properties of asbestos have encouraged widespread use of this mineral for centuries in a variety of applications. Unfortunately, prolonged exposure to airborne asbestos fibers adversely affects the respiratory system by reducing lung capacity and recent studies have related various forms of lung cancer to asbestos exposure. The Environmental Protection Agency (EPA) has moved to control emissions of asbestos because of its widespread use and hazardous nature [1][1]. The need to monitor airborne asbestos exists because of the difficulty in controlling all sources of emissions.

Respirable asbestos fibers range in length from a few micrometers (μm) down to submicrometer sizes. Median airborne asbestos fiber lengths reported in the literature range from about 0.5 to 5.0 μm, [2,4] and are best measured using electron microscopy. The EPA provisional methodology for airborne asbestos measurements [5] employs transmission electron microscopy (TEM) for identification and measurement of fibers collected on membrane filters. The method is an uneasy marriage of statistical sampling and TEM microscopy. The fibers are distinguished as asbestos by their morphology, crystal structure as determined by selected area electron diffraction (SAED), and their chemical composition as determined by x-ray fluorescence (XRF). Fiber count, length, diameter, and calculated mass are reported.

Samplers ranging from high volume (hi vol) with 8 x 10" (406.5 cm^2) filters to personal samplers with 37 mm (8.55 cm^2) diameter filters have been used to collect airborne asbestos. A 3 mm diameter circle is taken from these filters for analysis by TEM. The 3 mm circle is placed on a 200 mesh grid and up to 10 grid openings are examined according to a strict counting protocol. The grid openings are approximately 75 to 100 μm square (10^{-4}cm^2) and to relate the fibers on the grid to the original filter size multiplication factors between 10^2 and 10^5 are needed. The importance of a well defined counting protocol is obvious.

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[1] Figures in brackets refer to the literature references at the end of this paper.

Testing the counting protocol has been accomplished by repeated experimental observations by TEM which are tedious, time consuming, and expensive. This paper describes a computer simulation of sampling asbestos fibers and tests various protocols for counting and determining mass of the fibers. The simulation program was developed on the UNIVAC 1100 at the EPA NCC. The program uses IMSL [3] and Tektronix software and Tektronix hardware.

Steps in the simulation process:

1. Input all parameters pertinent to the simulation.

    a. Parameters for the bi-variate distribution of length and width of the filters. The program has been set up to handle four distribution types: normal, lognormal, three-parameter lognormal, and four-parameter lognormal (Johnson S_b).

    b. Fiber density and variation. The actual number of fibers on the filter is considered to be a normally distributed random number. The input fiber density is the mean of this number and the variation is the variance of this distribution.

    c. Filter size and shape. The shape of filter can be either circular or rectangular. The dimensions are defined by length and width or diameter. Also a limit is defined as to how close to the edge a plug can be taken for sampling purposes.

    d. Size of TEM grid openings and dimensions of the TEM field of view. The grid openings are considered to be square, and the field of view is considered to be rectangular.

2. Construct a seed for the various random number generators used in the simulation. This seed is based on the time of day the program is run and is therefore changed for every run since identical seeds produce identical sequences of random numbers.

3. Print out all parameters input to start the simulation.

4. Locate the 3.0 mm plug on the filter surface at a random point on the filter. The plug is located so that it will not be too close to the edge of the filter.

5. Orient grid openings on the plug. the grid is oriented at a random angle. The grid is simply defined as a series of coordinates corresponding to the grid opening size. There is no space between grid openings as there is on real TEM grids.

6. Select 10 grid openings at random. The 10 grid openings are selected at this point so that fiber information may be stored for sampling purposes later on. Only 10 are selected because no more than 10 can be sampled according to the provisional method.

7. Generate fibers for the entire filter.

    a. Generate length and width.

    b. Generate location and orientation on the filter. The fiber location is generated as a uniform random placement on the filter. The orientation is a random angle.

    c. Compare location with selected grid openings and store if necessary. The fiber location is generated at a uniform random placement on the filter. The orientation is a random angle.

    d. Accumulate statistics for fibers on the filter.

8. Print out statistics for fibers on the filter.

9. Make an initial count on 1st selected grid opening. Based on this count, follow procedures for medium or low loading (high loadings are not counted).

10. Print out statistics for fibers in the samples.

11. Compute errors of estimation in sample. Example of output can be found in Appendix A.

2. Conclusions

1. The aspect ratio cutoff of 3.0 causes bias in fiber count estimation and mass estimation. For six runs at low loading, the average percent difference between estimated and true fiber count was -34.44. The average percent difference for mass was 1.96. The large bias in count is expected due to the aspect ratios criteria. The lack of bias was not expected for the estimates of mass. One possible explanation for this is that most of the mass is concentrated in the higher fibers which have a higher probability of falling in the sample, whereas the smaller fibers and the larger fibers are counted the same.

   For five runs at medium loading, the average percent difference for estimated mass was -30.13 and the average percent difference for estimated count was -34.93. The mass has just as high a bias in this case. One possible explanation for this is that when sampling via the smaller field of view, the larger fibers will have a high probability of being truncated by the field of view; therefore, the estimate loses mass.

2. The border line between low and medium loading levels causes problems because not enough random fields of view can be found to get the required number of fibers in the sample.

3. We found that the smaller fields of view (< 5x5 μm) caused serious truncation of fiber length distribution, and on border line medium loadings the same problems of not being able to define enough random fields of view to take a large enough sample occurred.

2.1 Future Problems to be Addressed

The simulation program can be used to test different sampling protocols for increased precision, to characterize the effects of a clumped fiber distribution across the filter. The simulation will also generalize assumptions such as non-bending fibers and no operator error.

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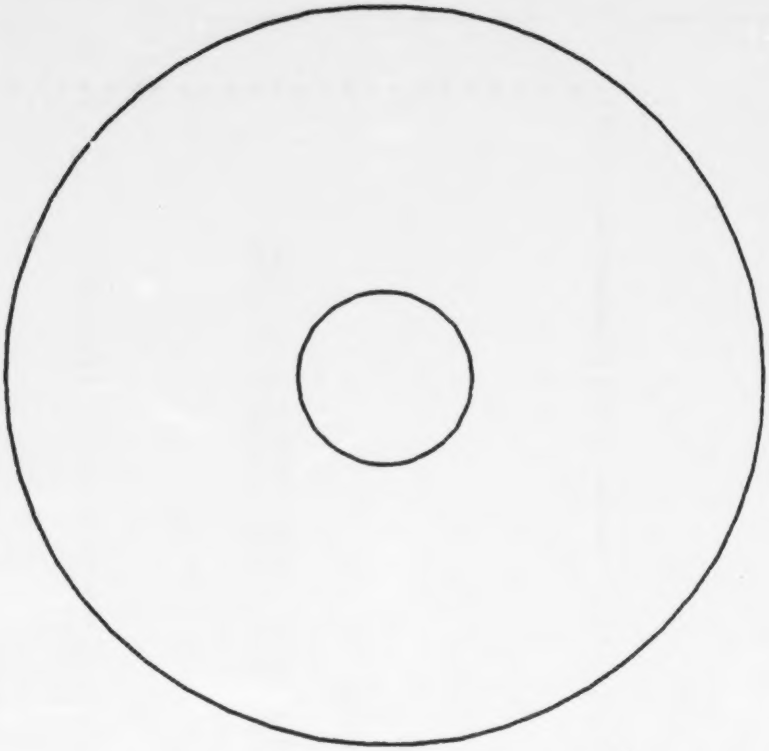
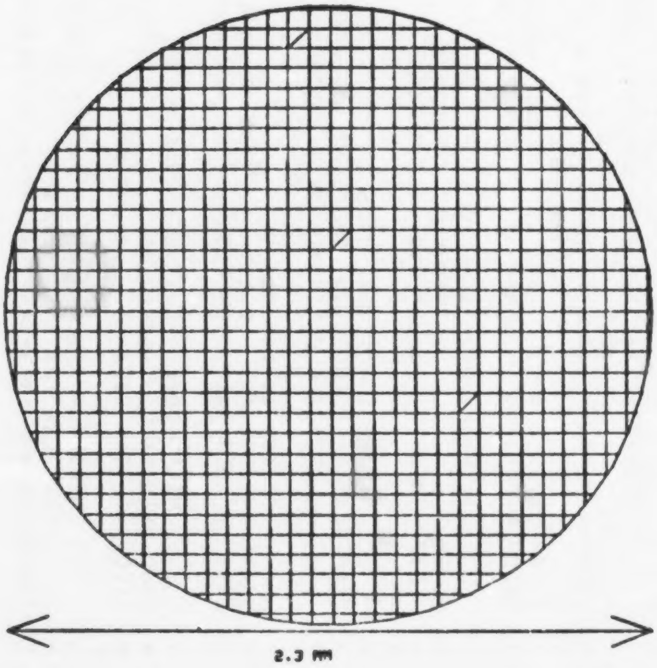
APPENDIX

EXAMPLE OF OUTPUT FROM SIMULATION PROGRAM

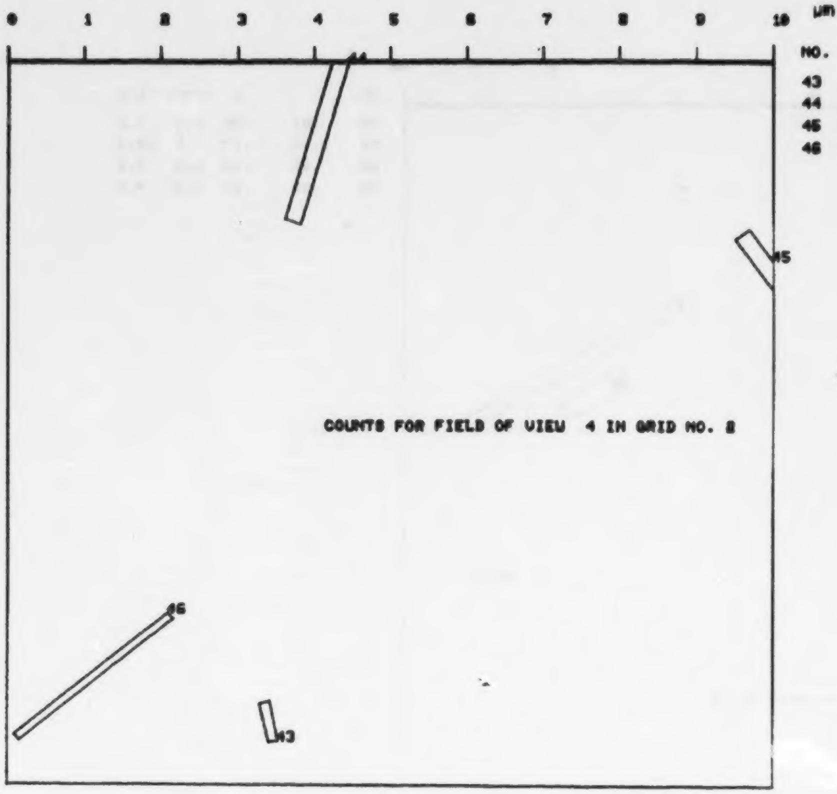
```
***********************************************************************************************************
*                                                                                                         *
*       INPUTS FOR SIMULATION :                                                                           *
*                                                                                                         *
*                                                                                                         *
*       FIBERS: BIVARIATE 2-P- LONORMAL DISTRIBUTION                                                      *
* MU L =          -.3000         MU W =         -1.9700                                                   *
* SIGMA**2 L =   1.4200          SIGMA**2 W =     .4000                                                   *
*                          COVARIANCE (L , W) =   .40000                                                  *
* LOWER BOUND L =       .00          LOWER BOUND W =       .00                                            *
* UPPER BOUND L =     .0000          UPPER BOUND W =     .0000                                            *
*                                                                                                         *
*       SCALING FOR FIBERS :                                                                              *
* MINIMUM L =       .00 MICRONS    MAXIMUM L =       .00 MICRONS   SCALE FACTOR =     .5500               *
* MINIMUM W =       .00 MICRONS    MAXIMUM W =       .00 MICRONS   SCALE FACTOR =     .0200               *
*                                                                                                         *
*       SAMPLE CHARACTERISTICS :                                                                          *
* MEAN DENSITY = 20000.00 FIBERS/MM      VARIANCE OF DENSITY =       .50                                  *
*          SPATIAL DISTRIBUTION :  UNIFORM                                                                *
*                                                                                                         *
*       FILTER DIMENSIONS :                                                                               *
* SHAPE : CIRCULAR     LENGTH =     10.00 MM WIDTH =     10.00 MM DIAMETER =     10.00 MM                 *
* LIMIT OF DISTANCE FROM EDGE A PLUG MAY BE TAKEN =      1.00 MM                                          *
*                                                                                                         *
*       MICROSCOPE CHARACTERISTICS :                                                                      *
* GRID OPENING =   .07500 MM               FIELD OF VIEW LENGTH =    .01000 MM                            *
* FIELD OF VIEW WIDTH =    .01000 MM                                                                      *
*                                                                                                         *
*       SEED FOR GENERATION OF RANDOM NUMBERS =     645715656                                             *
*       CUT OFF FOR ASPECT RATIO =  3.0                                                                   *
***********************************************************************************************************
```



Location of plug on the filter

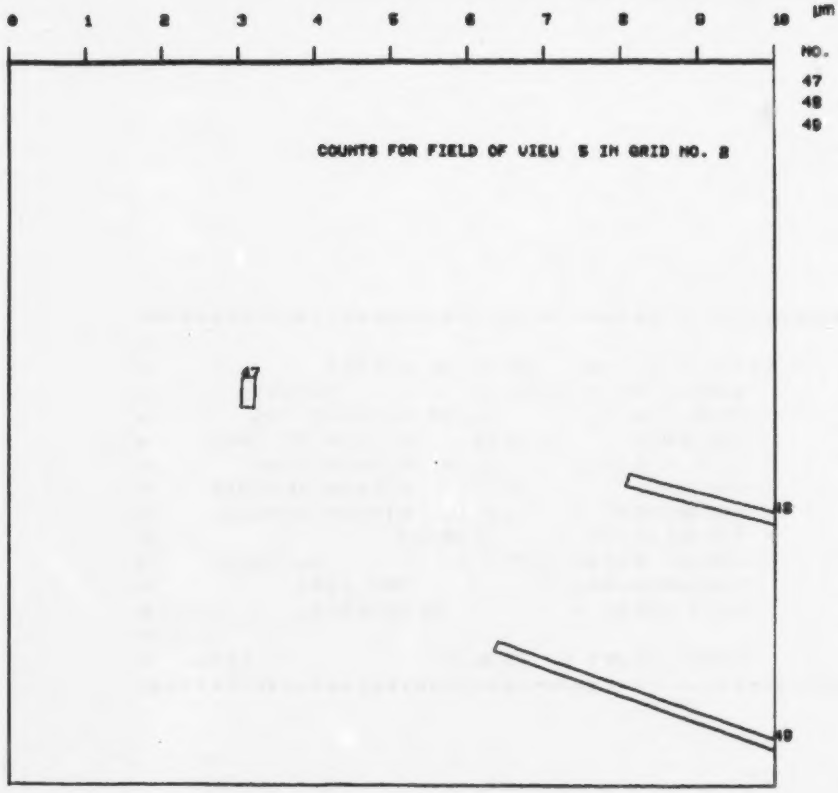


Grid opening selected for sample.



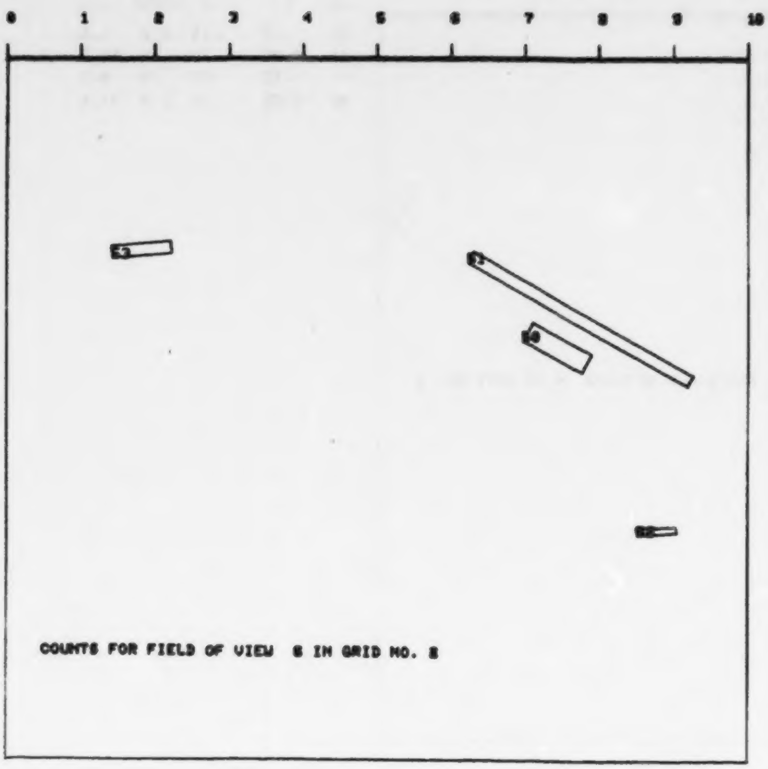
COUNTS FOR FIELD OF VIEW 4 IN GRID NO. 2

NO.	L	W	COUNT	L/W
43	.57	.13	1.0	4.5
44	2.29	.21	.5	10.7
45	.52	.22	.0	2.3
46	2.66	.11	1.0	24.2



COUNTS FOR FIELD OF VIEW 5 IN GRID NO. 2

NO.	L	W	COUNT	L/W
47	.42	.16	.0	2.5
48	1.95	.17	.5	11.5
49	3.87	.14	.5	26.9



COUNTS FOR FIELD OF VIEW 6 IN GRID NO. 2

NO.	L	W	COUNT	L/W
50	.91	.28	1.0	3.2
51	3.42	.17	1.0	20.4
52	.52	.10	1.0	5.2
53	.80	.17	1.0	4.6

```
*************************************************
*                                               *
*     STATISTICS FOR FIBERS ON FILTER           *
*       NUMBER OF FIBERS =          1570815     *
*       MEAN LENGTH =      1.59 MICROMETERS     *
*       VARIANCE =   8.9279    MICROMETERS**2   *
*       MEAN WIDTH =     .1604  MICROMETERS     *
*       VARIANCE =     .0087    MICROMETERS**2  *
*       COVARIANCE =     .0513  MICROMETERS**2  *
*       CORRELATION =    .184203                *
*       ASPECT RATIO (AVE) =        10.96190    *
*       VARIANCE(AR) =         392.7143         *
*       MASS (GMS) =        .0025119341         *
*                                               *
*       FIBER COUNT ON PLUG =           1198    *
*************************************************
```

```
     DISTRIBUTION OF FIBER LENGTH AND WIDTH
          ON FILTER
          IN SAMPLE

.00- .02        197       27       11        2        4        1        2        0        0        1
                  0        0        0        0        0        0        0        0        0        0

.02- .04      10749     2598      909      461      245      143      101       68       45      145
                  2        0        0        0        0        0        0        0        0        0

.04- .06      45605    14891     6070     3049     1720     1060      711      458      343     1255
                  4        1        1        0        0        0        0        0        0        0

.06- .08      78607    30402    13613     7201     4395     2662     1779     1279      935     3690
                  7        3        0        0        1        0        1        0        0        0

.08- .10      90267    40789    19193    10514     6475     4190     2853     2021     1504     6304
                  5        3        1        1        0        0        0        1        0        4

.10- .12      86356    43054    21158    12096     7571     4999     3488     2541     1809     8201
                  4        3        3        1        1        1        3        0        0        0

.12- .14      74122    40455    20933    12103     7670     5267     3732     2698     2002     9147
                  4        3        0        1        2        0        0        0        1        1

.14- .16      59451    35719    18870    11020     7146     4986     3512     2598     1998     9103
                  1        3        3        1        1        2        2        0        0        4

.16- .18      46792    29476    16013     9735     6569     4425     3251     2378     1799     8693
                  0        4        1        0        1        1        0        0        1        2

.18- .20     143668   108396    65593    42651    29054    20930    15529    11826     9168    51520
                  0        4        5        3        5        2        2        2        2        7

          .000  -  .550   - 1.100   - 1.650   - 2.200   - 2.750   - 3.300   - 3.850   - 4.400   - 4.950   - 5.500
                                Y - WIDTH VS. Y - LENGTH
                                ALL CLASSES DEFINED IN MICROMETERS
*********************************************
*                                           *
*    STATISTICS FOR FIBERS IN SAMPLE        *
*      NUMBER OF FIBERS =             198   *
*      MEAN LENGTH =     1.78 MICROMETERS   *
*      VARIANCE =   3.2926   MICROMETERS**2 *      *************************************************
*      MEAN WIDTH =    .1530  MICROMETERS   *      *        TOTAL AREA IN SAMPLE =  .008400000 MM**2 *
*      VARIANCE =    .0074    MICROMETERS**2*      *     TOTAL AREA IN FILTER =  78.5398 MM**2       *
*      COVARIANCE =   .0633  MICROMETERS**2 *      *        TOTAL COUNT FOR SAMPLE =    100.5        *
*      CORRELATION =   .406076              *      * TOTAL ESTIMATED COUNT FOR FILTER =    939672.8 *
*      ASPECT RATIO (AVE) =        12.14755 *      *        PER CENT ERROR =        -40.1793         *
*      VARIANCE(AR) =          157.6363     *      *        MASS IN SAMPLE =   .0000002035           *
*      MASS (GMS) =        .0000002035      *      *        ESTIMATED MASS ON FILTER  =     .001903 *
*                                           *      *        PERCENT ERROR =         -24.2478         *
*********************************************      *************************************************
```

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ANALYTICAL ERRORS IN ASBESTOS ANALYSIS BY ANALYTICAL ELECTRON MICROSCOPY

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Abstract

Instrument and operator related error can combine to yield large inaccuracies in analyzing asbestos samples by the analytical electron microscope. Imaging, diffraction, and mechanical stage problems can yield cumulative errors causing results of the same area of a sample to vary by a factor of two or more. Operator precision and bias vary considerably. The five operators for which the data was collected have an average tendency to undercount by approximately 10 percent or greater in the samples investigated (loadings of 25-45 fibers per 200 mesh grid square, using NIEHS short fiber chrysotile). Most of this undercounting occurs in the short, single fibrils that are under 0.4 µm in length. At or below this length our operators have approximately a 50 percent or less chance of detecting the fibers. Since our operators yield comparable results with other experienced laboratories, we expect that other operators are also missing small fibers.

1. Introduction

Measurement of asbestos in the ambient environment is currently accomplished using a filter collection procedure followed by an analytical electron microscope (AEM) analysis. A 1977 multilaboratory study of ambient air samples using unspecified preparation and counting methods gave results on split samples that varied by several orders of magnitude [1][1]. Since that time the Environmental Protection Agency (EPA) has been developing a standard methodology, but even using these techniques and the most experienced laboratories, interlaboratory results generally vary by a factor of two or more [2-4]. These differences may be due to a number of factors including variations in sampling, sample preparation (including filter homogeneity), instruments, and operators. Since most interlaboratory studies have used split samples and are currently using the same preparation methods, the variation should be due to instrument, operator, and homogeneity problems. The subject of this paper is the data collected on operator and instrument related error during the preparation of asbestos filter research materials at the National Bureau of Standards (NBS). The paper by Leigh et al., in these proceedings discusses filter homogeneity.

2. Sample Preparation

The filter research materials are prepared by filtering suspensions containing small amounts of National Institutes of Environmental Health Sciences short fiber chrysotile [5] and St. Louis Urban Air Particulate, NBS Standard Reference Material 1648, in filtered distilled water onto 0.1 or 0.4 µm filters. Indexed 200 mesh copper transmission electron microscope (TEM) grid specimens are prepared for AEM analysis using the EPA provisional method for analysis of asbestos in air [2].

---

[1] Figures in brackets refer to the literature references at the end of this paper.

3. Counting Procedures

In order to achieve the greatest accuracy possible, three to five operators were required to analyze each grid opening. Two of these operators had 5-6 years experience in asbestos analysis and the other three operators had accumulated 1.5 to 2 years experience.

Each grid square was traversed at a magnification of 20,000X. Each operator counted the square without major stage movement or changing samples between counts and also without discussion of the analysis with the other operators.

4. Instrument Related Errors

Eight transmission electron microscopes (TEM) were tested to find the instrument best suited to our analytical needs and to get an idea of the problems associated with instrumentation. These included TEMs from most of the major manufacturers. The instruments ranged in age from 25 years old to brand new and included both 100 kV and 200 kV models.

A number of the TEMs tested were found to have imaging and mechanical problems which increased the error in asbestos analysis. None of the TEMs in this study was used solely for asbestos work and all had multiple users with a wide variety of interests. Many of the problems encountered could and should have been corrected if the instruments were to be used largely for asbestos work. But this study was intended only as a survey to see the types and general magnitude of problems that could be expected in the field.

Imaging related parameters affecting asbestos analysis were found to include operating voltage, brightness, contrast, resolution, working magnification, and diffraction capabilities. Mechanically related errors appeared to be caused mainly by stage translation controls.

Under most circumstances the analysis of chrysotile on the TEM is best carried out at voltages of approximately 80-120 kV. At these voltages, chrysotile has good contrast with the carbon support film and the hollow tubular structure, if present, can be observed. At higher voltages this contrast can be considerably less and analysts can miss detecting fibers more easily. For amphiboles, contrast is less of a problem, and often higher voltages are required to attain diffraction patterns from thicker particles.

The brightness, contrast, and resolution of the TEMs used differed considerably. Some of the older instruments had considerably poorer images than the newer models, causing operators to miss up to 50 percent of the fibers as well as prematurely tiring operators due to the strain of trying to see and distinguish fibers. In addition, it was found that several older 200 kV TEMs operating at 100 kV often had insufficient brightness and contrast for easy observation of chrysotile. These problems were not encountered on the new 100 and 200 kV models. Half of the eight TEMs used, increased the error, the time of analysis, and operator fatigue due to poor image quality.

The diffraction conditions and data also varied greatly from instrument to instrument. On some of the older instruments less than 10 percent of the chrysotile fibers yielded good selected area diffraction patterns visible to the operator. On instruments that were about three years old or newer, greater than 90 percent of the chrysotile showed good selected area diffraction (SAD) patterns. This disparity is probably due to a number of reasons including the longer time needed to set up SAD conditions in the older instruments, the efficiency of the electron optics, phosphors, etc., and the fact that chrysotile's crystallinity is electron dose dependent.

Magnification also plays an obvious and important role in both the efficiency and accuracy of asbestos analysis. At 20,000X a single chrysotile fibril 30 nm wide appears to be approximately 0.6 mm wide with a hollow tube ~0.1 mm wide. This fiber width is reasonably easy for the human eye to see and the hollow tube can easily be observed by using a binocular microscope. At magnifications lower than about 10,000X these thin fibers can be difficult to see and are often missed because they are only a few tenths of a millimeter wide. At these lower magnifications it is also more difficult to distinguish chrysotile from filter artifacts and other non-asbestos particles, so that more time is taken analyzing fibers that are readily distinguishable at 20,000X. At magnifications of 30,000X and higher more traverses are needed to cover a TEM grid square and the area and time of analysis increases by about 2 1/2 times. In addition any stage traverse error (see below) will be likewise amplified. Therefore, for reasons of economy and accuracy we have found the optimum magnification for scanning grids for chrysotile is approximately 20,000X.

The major mechanical problem associated with the TEMs was found to be the mechanical stage. Stage movement stability and reproducibility is extremely important. During the analysis of asbestos a grid square approximately 80 µm on a side is traversed at magnifications of about 20,000X. Any wandering of the stage during a traverse can cause areas and fibers to be missed or reanalyzed. Some of the stages erratically wandered by as much as 30 µm off a straight line traverse, causing asbestos counts to vary radically (over a factor of 2 difference on the same square). Most stages have some "backlash" when traverse directions are changed, but these often are reproducible and can be corrected by transversing past the grid square and then back to it before starting the next traverse. The microscope used for the data collected on operator error in this paper has a stage which wanders <1 µm during a 100 µm traverse and is very consistent and reproducible in its actions.

5. Operator Error

Operator error was looked at once the machine error was controlled and defined. A variety of methods for reporting and comparing data have evolved during preparation of the filters. Initially, to determine the extent of operator error, each operator only reported the number of fibers in the same grid square. These initial values showed sporadic variations of over 50 percent. Some of this variability was found to be due to machine related problems while some was related to individual operator errors. In one effort to reduce this error and correlate the operators' counts, a counting data sheet was designed. This data sheet was made to mimic an operator's thought pattern during chrysotile counting. Fibers that were detected during the traverse of the grid square were catalogued by the following parameters:

- the traverse number in which the fiber was found
- whether a fiber was characterized by distinct, poor, or no hollow tube structure
- whether chrysotile's characteristic electron diffraction pattern was observed
- whether the ends of the fibers were visible or unobservable due to obstruction by a grid bar or another particle
- whether the fiber was a single fibril or a bundle of one or more fibrils
- and lastly whether the operator counted a fiber as a chrysotile fiber or as another type of particle.

The purpose of the chart was to identify which parameters have a significant effect on operator error and to determine operator variability and bias.

It was noticed that one operator had a consistently higher deviation from the mean than did the other operators. Table 1 shows the average of the absolute percent change from the mean for 30 grid squares by operator. The asbestos counts for operator 3 have averaged 15.6 percent higher or lower than the mean count for the five operators. Normalizing to the low deviation values of 6.9 percent of operators 2 and 4 shows that counter 3 had an average deviation from the mean over 2 times greater than that for operators 2 and 4.

Table 1. Tabulation of the Average Absolute Deviation from the Mean Grid Square Count in Percent by Operator.

Operator	1	2	3	4	5
Average percent deviation	10.9	6.9	15.6	6.9	12.0
Normalized	1.6	1.0	2.3	1.0	1.7

A "high variance" operator needs to count more grid squares and collect more data to obtain an equally reliable asbestos concentration. In a field laboratory with only one operator, the filter may be erroneously blamed for the large variation. In either case, a statistical analysis should give reasonable error bars. However, if an operator is consistently lower or higher than the mean, the bias will only be detected when compared to several other counters and would not be seen on the statistical analysis carried out on the data of a single counter (as is usually the case for commercial laboratories). Initially, the problem of operator bias was looked at qualitatively. For each data set, a ranking value of one through five was assigned to the lowest through the highest counter, respectively. These values for all the counts were summed and averaged per operator to determine his average placement or ranking. Table 2 shows such a tabulation. If there were no consistent bias by any of the operators, each operator's rank would be about three. It can be seen from Table 2 that counter 1 has a tendency to be low while operators 3 and 5 have a slight tendency to be high. The total number of high and low counts is also shown for each counter. Operator 1 has a large number of the lowest counts, while operator 3 has a large number of both the lowest and highest counts due to this counter's high variance.

Table 2. Average Ranking of Operators for Thirty Grid Squares and the Number of Low Counts and High Counts by Operator.

Operator	1	2	3	4	5
Ranking	2.0	3.0	3.4	3.0	3.4
# lows	11	1	8	3	3
# highs	1	2	14	3	8

An attempt was made to cross reference the counts made by the five operators to discover the cause or causes of the variation and bias. It was found that only the longer fibers could be cross referenced and it became obvious that all operators were not counting the same fibers. To prove this and to quantitate what the operators were counting, a system was devised that accurately located each fiber observed by each operator.

A digital voltmeter was put on the AEM's x and y specimen position controls. This gives the operator the capability of recording the stage position within approximately a micrometer. Each counter records the position of a fiber as well as the other data discussed above directly into a computer. Later, after all the operators have analyzed the grid square, a map is made (figure 1) which shows the position of each fiber for each operator. The fiber count on this grid square is then verified by having an operator reanalyze any fibers on which there were questions. This includes any fibers that were found by only one operator, or any fibers whose identification is disputed because of ambiguous morphology or diffraction data. In this way, a count approaching as closely as possible to the true amount of asbestos is derived. Prior to the cross referenced and verified counting method we used the average value as the number of fibers in the grid square. Table 3 compares the individual and average operator counts with the verified counts. Thus far, the verified counts have been greater than or equal to the average counts.

Morphology, diffraction, and sometimes chemistry determined with an energy dispersive x-ray detector are used to positively identify chrysotile. Because of beam damage to the crystallinity of the chrysotile only the first operator in our repetitive counting technique uses electron diffraction. This means that the other operators, using only morphology, are somewhat more apt to record false negatives and false positives than a field laboratory that used diffraction. This is especially true of the false positives which should be almost non-existent, since none of the fibrous minerals with diffraction patterns similar to chrysotile have been found in our samples. Table 4 shows the breakdown of the errors. Most of the chrysotile fibers that were not counted were simply missed, i.e., only a small number were misidentified as the other. Almost all of the false negatives were fibers which showed poorly defined or no hollow tube structure and were placed in the "other" category due to lack of diffraction data.

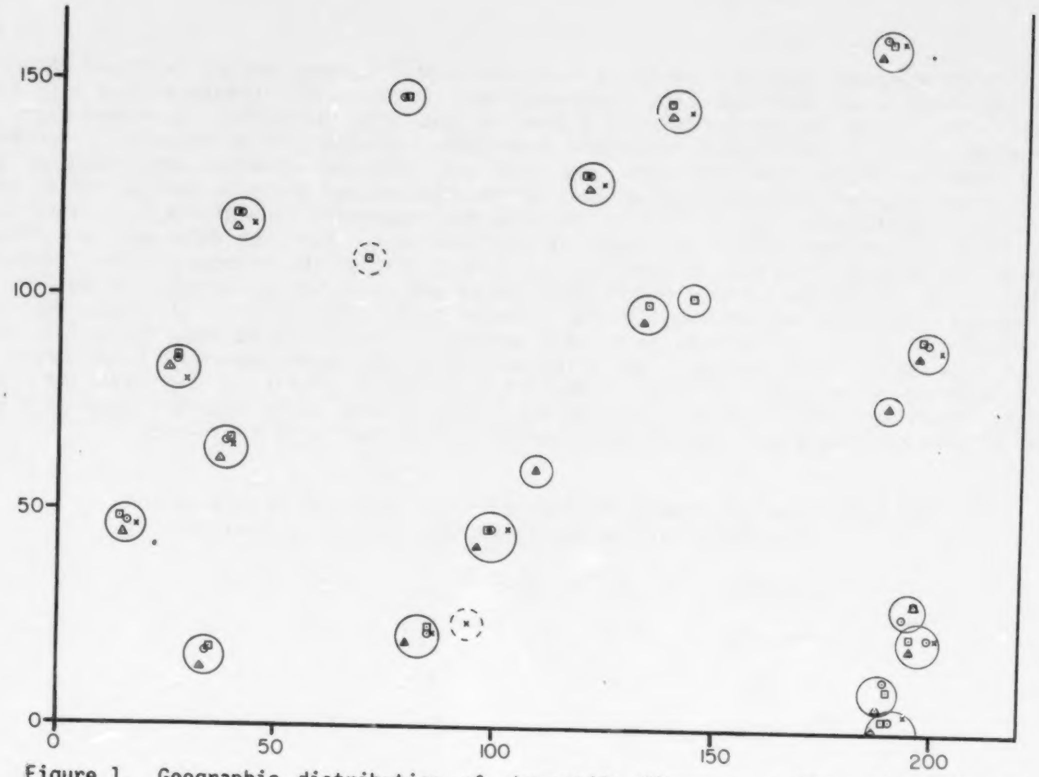

Figure 1. Geographic distribution of chrysotile fibers on a TEM grid square as analyzed by four operators. The small circles, triangles, squares, and x's represent individual chrysotile counts for each operator. The large solid circles are verified chrysotile fibers and the dashed circles are verified "other" particles originally counted as chrysotile.

Table 3. Comparison of Operator Counts and Mean Counts to Verified Count on 75 μm x 75 μm Grid Opening.

Grid / Operator	1	2	3	4	5	Mean Count	Verified Count
1	20	19			17	18.7	20
2		22		18	21	20.3	24
3		21		13	21	18.3	21
4	30	24	21		23	24.5	29
5	24	31	25		25	26.3	31
6	18	17			19	18.0	18
7	24	23			25	24.0	29
8	24	22	18		27	22.8	24
9	22	20	27		20	22.3	24
10	16	19	21	21	20	19.4	22
11	19	16	13		18	16.5	20
12	19	18	20		14	17.8	20
13	31	24	20		25	25.0	26
14	26	19			21	22.0	22
15	27	28	28		30	28.3	30

Table 4. Percent Deviation from the Verified Counts by Operator.

Operator	A	B	C	D
Percent Fibers Not Observed	-10.62	-11.20	-29.27	-9.38
Percent False Negatives	-2.95	-1.30	-0.81	-3.13
Percent False Positives	+7.08	+1.82	+14.23	+5.21

One factor correlating with operators not observing fibers is fiber size. Figure 2 shows a graph of the fraction of operators finding fibers of a given length. The scatter of the points is too large to get a well defined fit, but the trend toward fewer operators seeing the smaller fibers is apparent. Once the chrysotile fiber length is below approximately 0.3-0.4 μm our operators have at best a 50 percent chance of finding it. This is probably true for other operators as well.

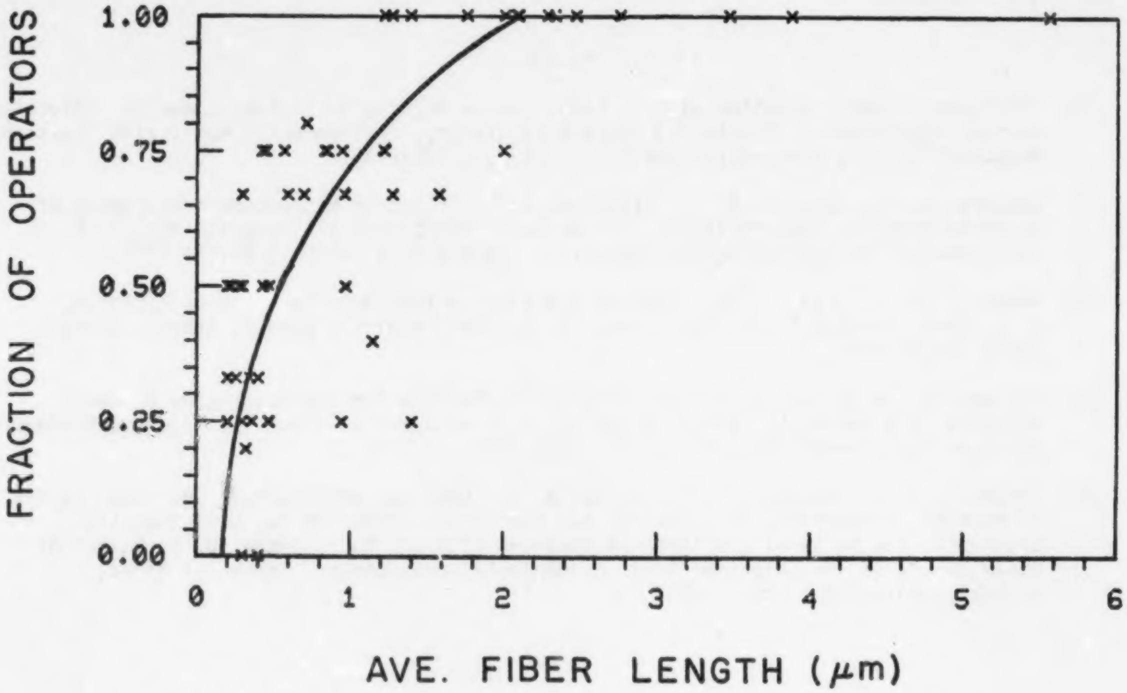

Figure 2. Fraction of operators observing a given length chrysotile fiber (averaged for each grid square).

The other major error was associated with the way each operator counted fiber bundles. Our samples contained very few complex bundles of chrysotile fibers, and even though all the operators used the same algorithm for counting bundles there were enough ambiguous cases to account for up to approximately a 5 percent error. On samples that contain large percentage of complex asbestos fiber bundles, such as some occupational samples, one may expect much larger errors due to operator differences in counting the bundles.

6. Conclusions

Machine related error can account for as much as approximately 100 to 200 percent variation in chrysotile grid counts. Only instruments with excellent imaging and precise stage controls should be used. Five out of the eight instruments used in this study were considered unusable for routine asbestos analysis because of deficiencies in image quality, diffraction capability, and/or stage movement precision.

Accuracy and precision vary considerably from operator to operator. This variation is not necessarily consistent through time, on the first filter operator 1 showed a definite bias, displayed as low counts, when compared to the other counters. On the second filter this relative bias was not observed. Among the five operators used in this study, the average variation for each operator ranged from approximately 7 to 16 percent of the mean number of fibers counted. All of the operators have a definite tendency to undercount fibers. Most operators failed to observe approximately 10 percent of the chrysotile fibers in our samples, but one operator missed an average 29 percent. The majority of the fibers missed are short single or double fibrils below approximately 0.4 μm in length. Below this length, our operators have approximately a 50 percent chance or less of observing the fiber. In round robin tests our operators have had comparable results to other experienced laboratories, thus implying that operators outside of this NBS study can be expected to demonstrate similar biases. Variation in counting the number of fibers in bundles is the second most common source of error among our counters.

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STATISTICAL CONSIDERATIONS IN THE PREPARATION OF CHRYSOTILE FILTER REFERENCE MATERIALS: FILTER HOMOGENEITY

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Abstract

The data discussed shows that asbestos fibers can be deposited in a manner compatible with a Poisson statistical process when using careful liquid filtration onto Nuclepore filters and when using a fiber density of approximately 10-70 fibers per grid square. However, this hypothesis should be tested for each filter using methods similar to those described. The analysis also shows that such filters can be homogeneous enough to allow individual sections of a filter to be considered as representative of the whole filter sample.

Note: In order to adequately describe materials and experimental procedures, it was occasionally necessary to identify commercial products by manufacturer's name or label. In no instance does such identification imply endorsement by the National Bureau of Standards nor does it imply that the particular product or equipment is necessarily the best available for that purpose.

1. Introduction

Measurement of asbestos in the ambient environment is currently accomplished using a filter collection procedure followed by an analytical electron microscope (AEM) analysis. A 1977 multilaboratory study of ambient air samples using unspecified preparation and counting methods gave results on split samples that varied by several orders of magnitude [1][2]. Since that time the Environmental Protection Agency (EPA) has been developing a standard methodology, but even using these techniques and the most experienced laboratories, interlaboratory results generally vary by about a factor of two [2-4]. These differences may be due to a number of factors, including variations in sampling, sample preparation (including filter homogeneity), instruments, and operators. Since most interlaboratory studies have used split samples and are currently using the same preparation methods, the variation should be due to instrument, operator, and homogeneity problems. The subject of this paper is the data collected on filter homogeneity during the preparation of asbestos filter research materials at the National Bureau of Standards (NBS).

2. Sample Preparation

The filter research materials are prepared by filtering a suspension containing small amounts of National Institutes of Environmental Health Sciences short fiber chrysotile and St. Louis Urban Air Particulate, NBS Standard Reference Material 1648, in filtered distilled water onto 0.1 or 0.4 µm Nuclepore filters [5]. Indexed 200 mesh copper transmission electron microscope (TEM) grid specimens are prepared for AEM analysis using the EPA provisional method for analysis of asbestos in air [2].

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[2] Figures in brackets refer to the literature references at the end of this paper.

3. Counting Procedures

In order to achieve the greatest accuracy possible, three to five operators were required to analyze each grid opening. Two of these operators had 5-6 years experience in asbestos analysis and the other three operators had accumulated 1.5 to 2 years experience. All the data reported in this paper was collected on either a JEOL 100CX or JEOL 200CX TEM operating at 100 kV and approximately 20,000X magnification. Additional counting information is contained in the paper by Steel et al., in these proceedings.

4. Statistical Analysis of Fiber Distribution Data

The validity of the common Poisson assumption regarding the underlying distribution for grid counts in the range of loading densities tested, the question of homogeneity of the fiber counts over the surface of a filter, and the accuracy to be anticipated in counting filters prepared to uniform loading density specifications are discussed. This information is based on the results from one trial filter (five operators counting, 0.1 µm Nuclepore) and one uniform filter (three to five operators counting, 0.4 µm Nuclepore).

The overall objective of the NBS research filter, and ideally of filter samples collected in the field, is to be able to control the process of fiber deposition and analysis to such a degree as to be able to measure the average count densities to within some preassigned relative error, where by relative error we mean an estimate of error – e.g., standard deviation – divided by the best estimate of count average. We replicate at two levels during the analysis procedure. We replicate within the grid square by having more than one operator count each square and across grid squares by analyzing many randomly chosen filter areas (grids) and grid squares.

5. Distribution Assumptions

It is easiest to characterize a process statistically if that process is one characterized by random sampling from a fixed (though not necessarily known) distribution with fixed mean and fixed variance. The classical assumption in many types of counting situations is that the underlying distribution for the counts is Poisson [6]. In these cases two implicit underlying assumptions are made: randomness and independence. In this context randomness refers to an "unpredictability" in the number of fibers deposited on any one cell of the counting grid. Independence means that no physical interaction is occurring among the chrysotile fibers during the deposition process.

Assume a series of grid counts, (x_i), has been obtained for a given filter. If the Poisson assumption is met for the filter, then the Poisson parameter, λ, as estimated by the mean count $(\bar{x})$, provides the best single summary of the filter's loading density. For such a Poisson filter the reported density will be

$$\bar{x} \pm \sqrt{x/N} \tag{1}$$

where N is the number of grid squares counted.

As a point of reference, typical contemplated NBS filter loading densities would be approximately 10-100 fibers per grid square. Figure 1 depicts the Poisson coefficient of variation $(\sqrt{\lambda}/\lambda)$ for different λ (loading densities) within this range. This curve is only a limiting curve on the relative errors obtainable for filter counts following these distinct Poisson processes in the sense that given a filter whose count distribution is known to be Poisson, of known λ, with the true counts on all of the grid squares known, then the relative standard deviation for the filter (the population relative standard deviation) will be the figure read off the curve. This curve is not, however, to be interpreted as saying that we cannot get as arbitrarily close as we like to the true λ in our estimation.

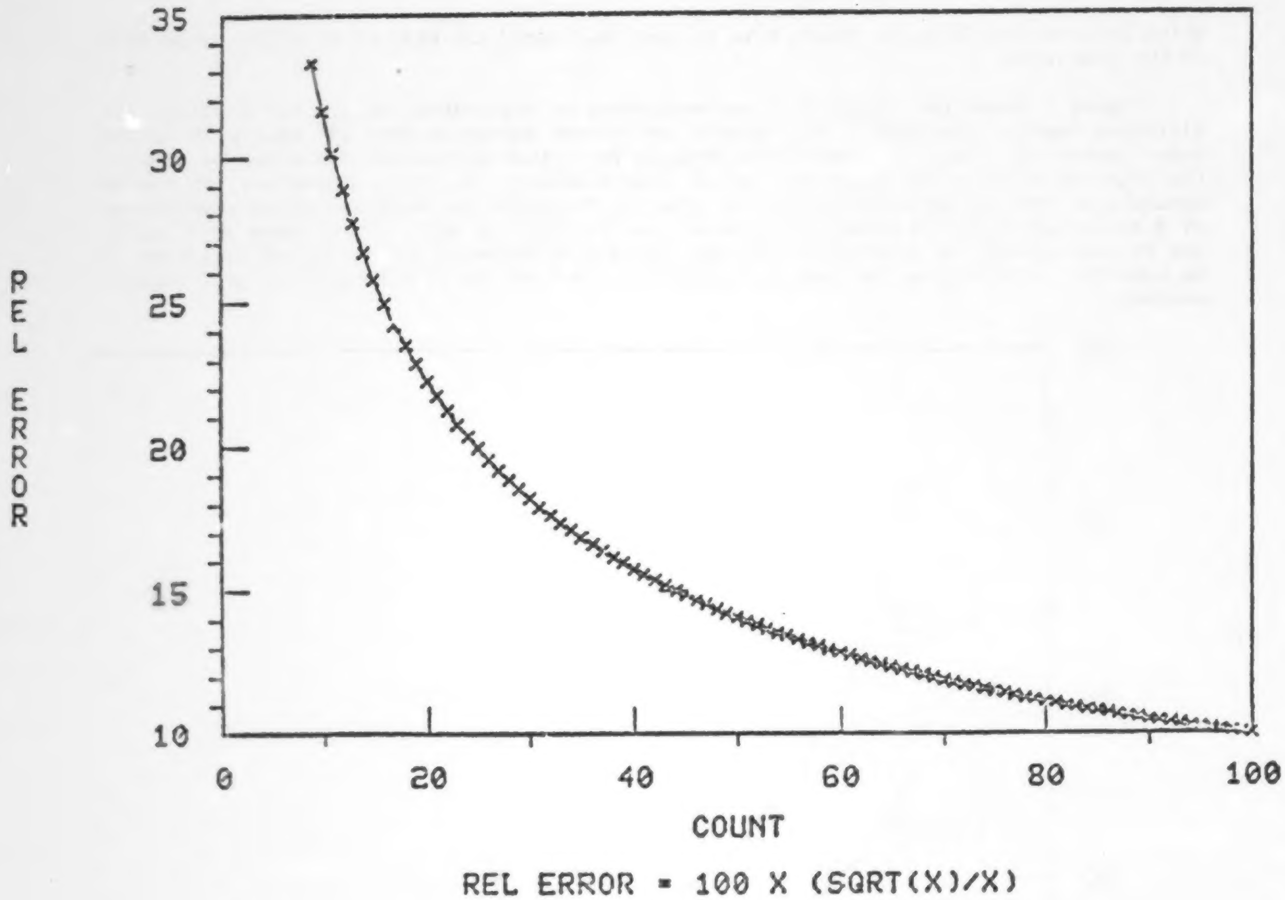


Figure 1. Poisson coefficient of variation.

We can in fact get arbitrarily close to the true λ by sampling at a sufficiently high frequency (N). In fact, if we can assume Poisson, then for prespecified maximum allowable uncertainties p in our estimates of λ, a very simple approximate relationship can be derived which specifies exactly how large N should be to keep the relative error below p. If p equals the maximum allowable uncertainty (specified in decimal terms, e.g., 0.10, 0.15, etc.) in our estimate of λ and is fixed, then by the standard formula for the sampling variation of the mean (ignoring finite population correction factors)

$$\frac{2(S/\sqrt{N})}{\bar{x}} < p \tag{2}$$

where the factor 2 is chosen because two standard deviations translates approximately into 95 percent confidence. This can be written

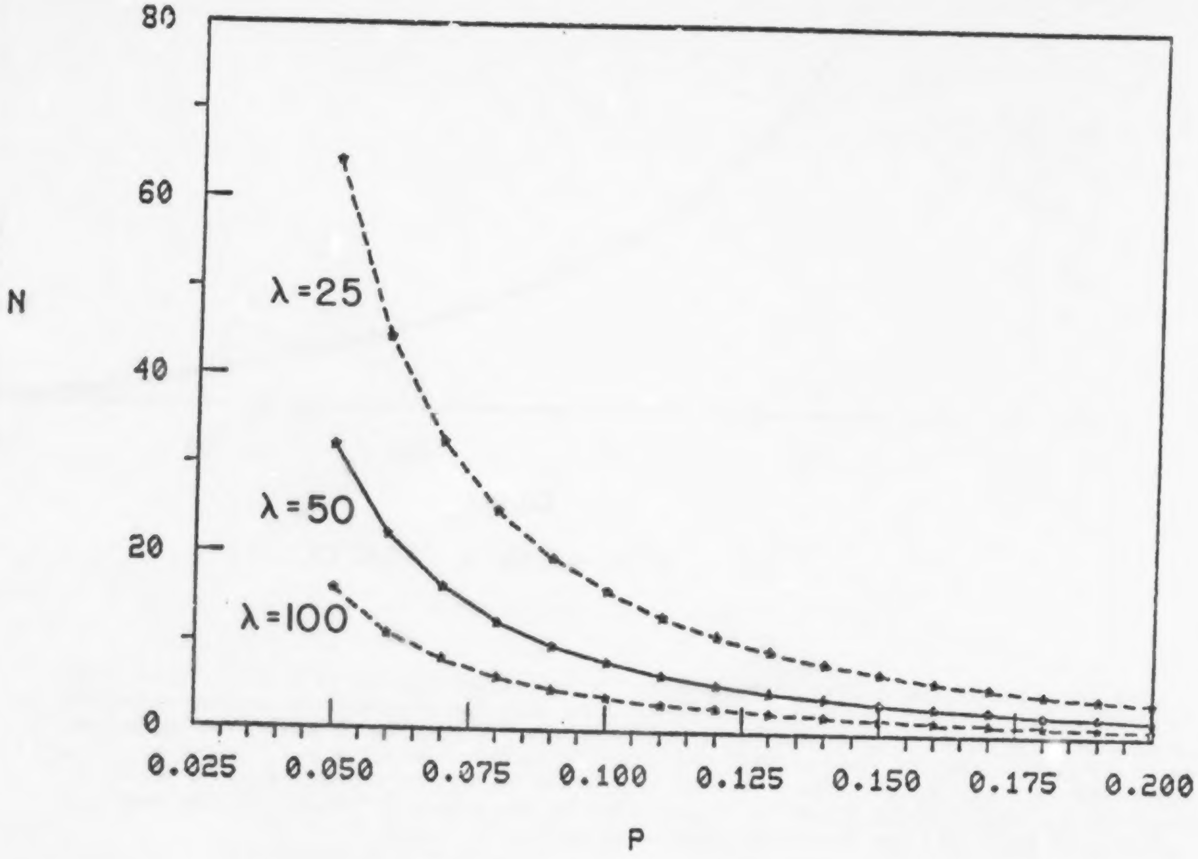
$$N \geq \left(\frac{S}{(p/2)\ \bar{x}}\right)^2 \tag{3}$$

Under the assumption of Poisson $\bar{x} \cong \lambda$ and $s \cong \sqrt{\lambda}$. Therefore

$$N\lambda > 4/p^2 \tag{4}$$

which tells us how large to choose N to get our (x_i) based estimate of to within ±p percent of the true value.

Figure 2 shows the ranges in N corresponding to reasonable choices for p values for different loading densities. The formula and curves emphasize that ultimately it is the total number of fibers counted – the product Nλ – that determines how close we come to the true value of λ for a filter, as we should expect. In field situations, it may be possible to vary the dilution or time of sampling to attain the most convenient combination of N and λ such that the product is greater than or equal to $4/p^2$. Thus, where applicable, the Poisson assumption provides a succinct formula determining the degree of precision to be expected in estimating the density parameter as a function of the number of grid squares counted.



LOADING DENSITIES (TOP TO BOTTOM) 25,50,100

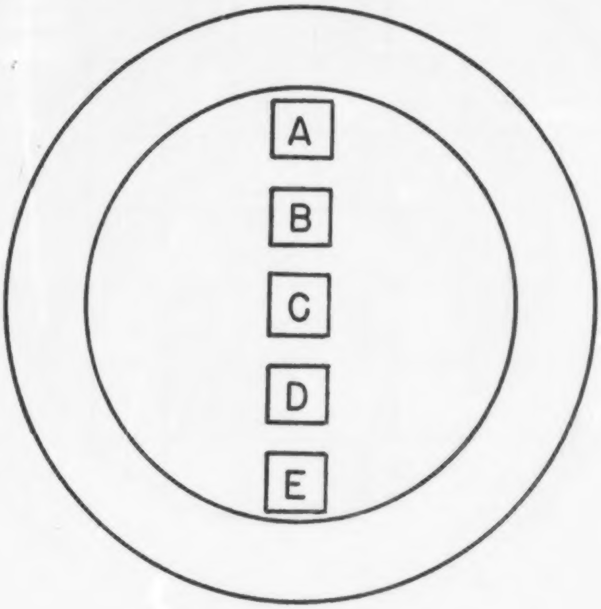
Figure 2. N for maximum allowable uncertainty in estimate of λ.

In fact, experience at some other laboratories suggests that at low and medium loading densities – the 10 to 70 fibers/75 μm² grid square range – a Poisson distribution of the counts obtained is to be expected, but at higher ranges the empirical count distributions may in fact not be explainable by Poisson. At these higher loading densities, fibers may not be acting in an independent fashion due to clumping or some other process during deposition. Thus one of the Poisson assumptions, independence, would not hold. But alternative distributions – such as the negative binomial or various compound or generalized distributions used for modeling clustered spatial distributions – may be suitable under these conditions [7]. For these reasons it is essential that any assumption of Poissonness or any alternative model for the distribution of fibers on the filter be tested.

One important limiting factor on all of the checks that were done for Poissonness (likely to be even more true for commercial laboratories with greater time restraints) is the small amount of data available. Twenty-five areas on the first trial filter and 38 areas on the second filter were analyzed. This precludes doing certain available checks of Poissonness, such as a simple graphical test described by Hoaglin [8].

Classical distributional tests that are available and were used include comparison of moments, chi-square goodness of fit, Kolmogorov-Smirnov, and probability plotting.

Figure 3 shows the distribution of the counts over the indicated surfaces on the first filter. The numbers listed for each lettered area are the average counts for five grid squares counted by five operators each.



A	50,51,53,53,39
B	45,37,41,53,49
C	43,44,40,50,51
D	43,26,46,47,54
E	44,47,42,56,44

Figure 3. Schematic of chrysotile counts collected on the first uniform 47 mm Nuclepore filter.

Figure 4 shows the actual occurrence pattern of the counts over the surface of the second filter. The picture is a composite, combining counts based on averaging over 3-5 operators counting the same grid square with count values based on the verified method (starred values) described by Steel et al., in these proceedings. This is done, because, where available, the verified data do in fact represent the best estimates for the true grid square counts, while where such values are unavailable the averages over the number of operators represent the best estimates of "true" counts.

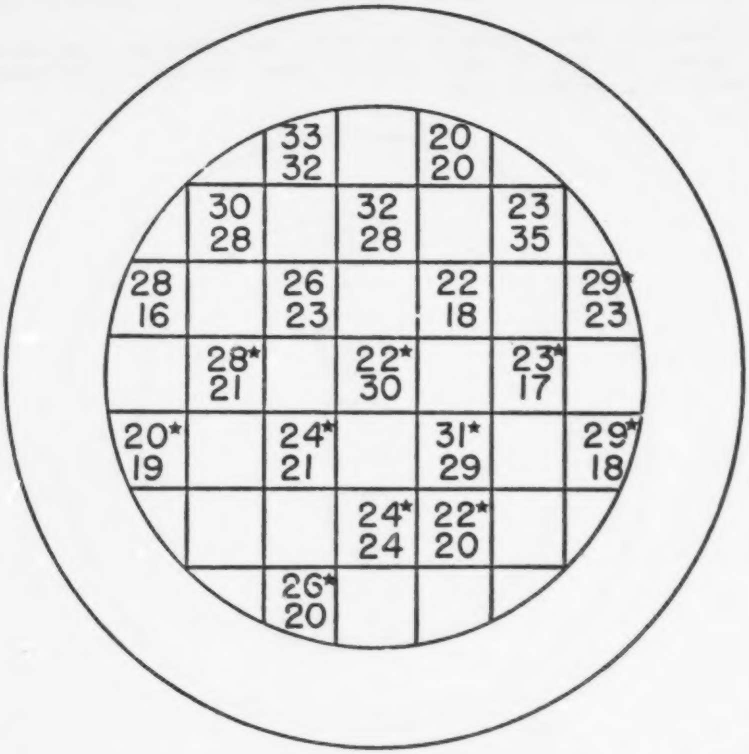


Figure 4. Schematic of chrysotile counts collected on the second uniform 47 mm Nuclepore filter. Each square represents a filter section from which a TEM specimen grid was prepared. The numbers are the chrysotile counts from two grid squares in each of the grids analyzed.

Figures 5 and 6 show histograms of the counts obtained on the two filters. Aside from an apparent biomodality in each, both distributions look reasonably symmetric and normal-looking - as they should for loading densities above approximately 10-11 fibers per grid square.

Comparison of moments, shown in Table 1, show reasonable agreement with theoretically predicted variance for the first filter and good agreement in both second and third moments for the second filter. On the second filter the mean does in fact equal the variance out to four significant figures (0.2459) - as it should for Poisson distributed data - and an actual skewness coefficient of 0.245 compares favorably to a Poisson-predicted $\lambda^{1/2}$ of 0.202. Fourth moments (kurtosis) do not compare as well, possibly because of the valleys in the middles of the histograms.

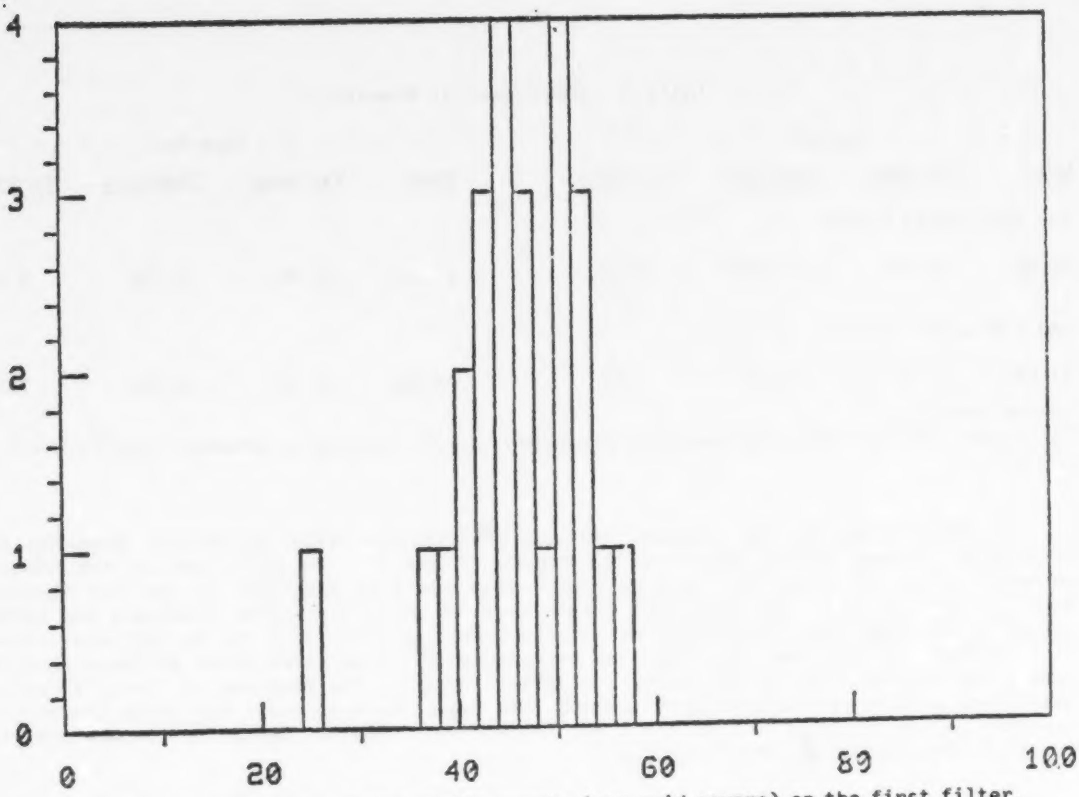


Figure 5. Histogram of chrysotile counts (per grid square) on the first filter.

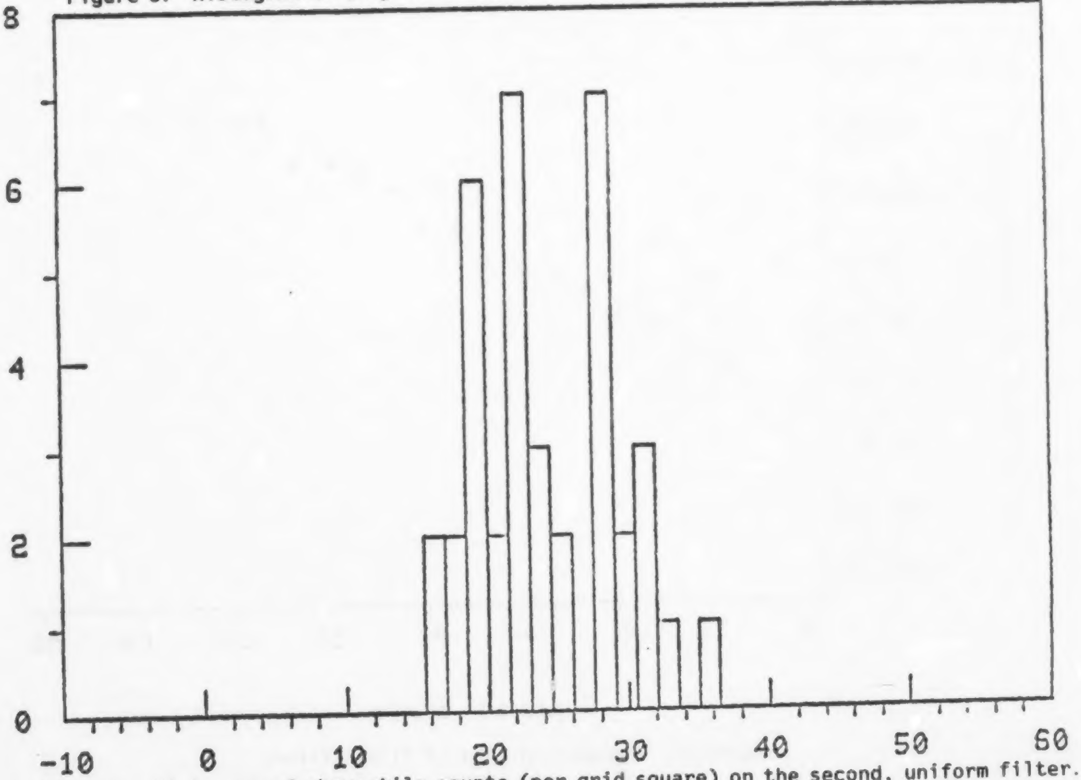


Figure 6. Histogram of chrysotile counts (per grid square) on the second, uniform filter.

Table 1. Comparison of Moments.

Actual				Expected			
Mean	Variance	Skewness	Kurtosis	Mean	Variance	Skewness	Kurtosis
1st (25-count) Filter							
45.86	43.19	-0.976[a]	4.39	45.86	45.86	0.148	3.02
2nd (38-count) Filter							
24.58	24.57	0.245	1.98	24.58	24.58	0.202	3.04

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[a] If censor the one outlying count (cf Histogram) of 26, compute a skewness (coefficient) of -0.08.

Probability plots for assumed Poisson distribution with parameters equaling the respective average counts are shown in figures 7 and 8. The plots are of the ordered observed counts vs. "typical" ordered counts that would be predicted if the distributions were in fact Poisson. The predicted values used in these particular plots are the median values of the sampling distribution of the ith order statistic from the appropriate Poisson distribution [9]. If the resulting plot is reasonably linear it provides evidence that the underlying distribution of the counts is indeed Poisson. The nearness to linearity can be quantified with a correlation coefficient. The two plots here both look quite linear with correlation coefficients of 0.95 and 0.98, respectively. Again, censoring the one outlying count of 26 would improve the first plot.

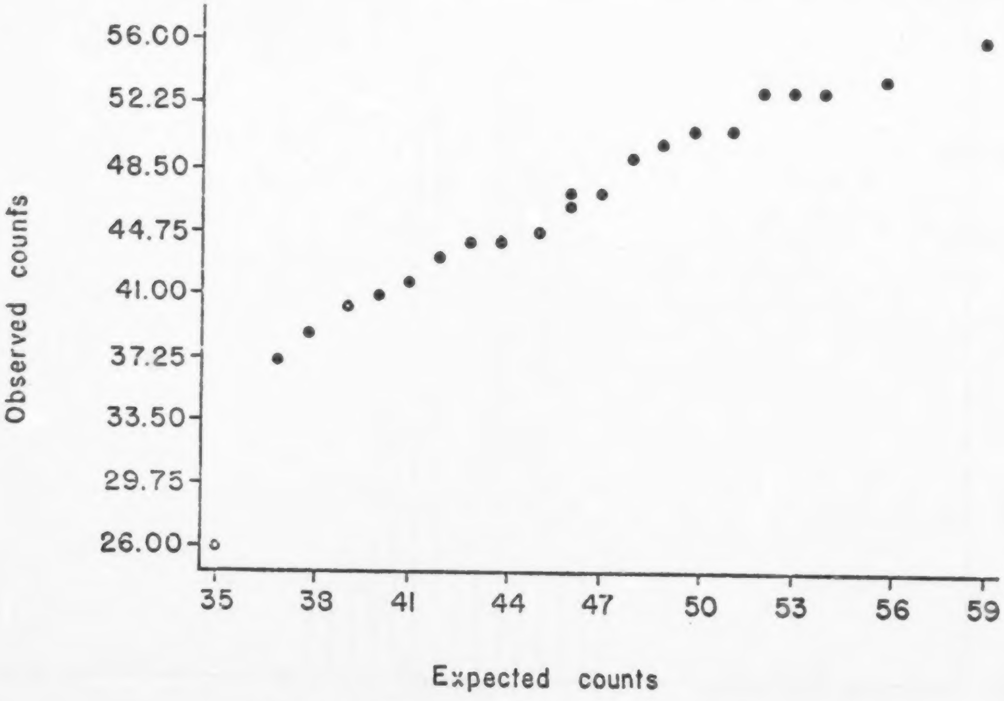

Figure 7. Probability plot of first filter.

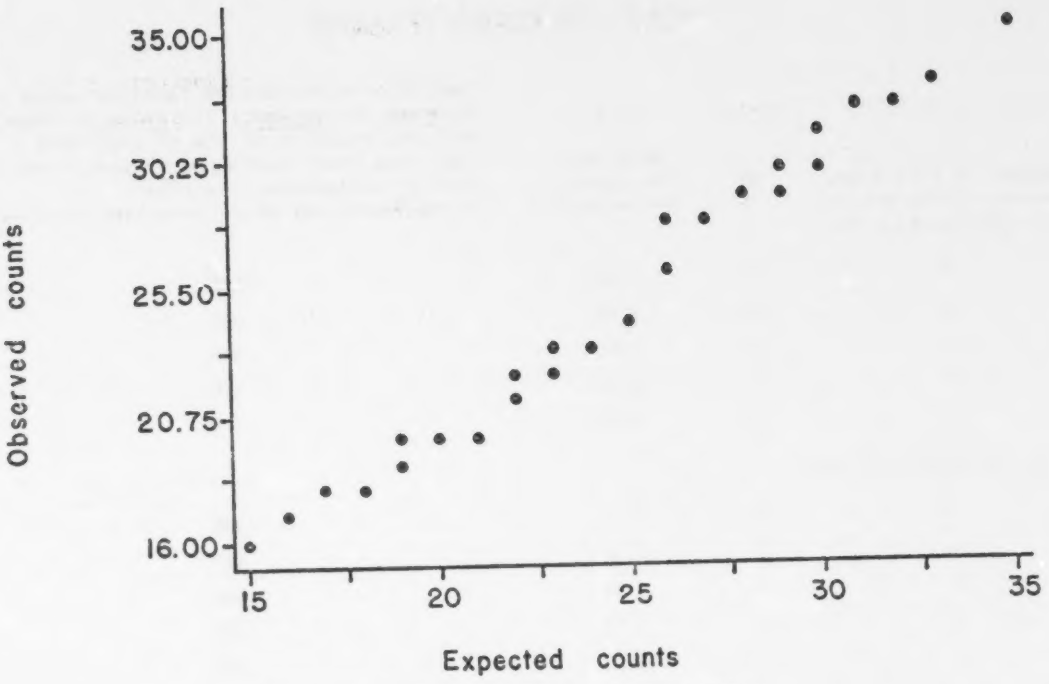

Figure 8. Probability plot of second filter.

Equiprobable category chi-square goodness-of-fit tests were done for each filter, the results of which are shown in Table 2 and figure 9. Equiprobable partitioning is used here because of the relatively small number of counts available. Equiprobable partitioning means choosing intervals over the range of count values such that the theoretically expected values (of occurrence of count values in that range) are the same. This is done to avoid inflated contributions to the chi-square statistic from zero count cells and to avoid weighting the smaller expectation values occurring in the tails of the distribution. In all instances - each of the five equipartitions of each filter - the test is seen to be compatible with an assumption of Poissonness.

Kolmogrov-Smirnov (one sample) test of goodness-of-fit was performed and also confirmed the compatibility of the frequency distribution of counts with Poissonness for the two filters as shown in Table 3.

Table 2. Equiprobable Chi-square.

Number of Partitions	df	Observed Chi-Square	Cumulative distribution function value of observed chi-square. The value is from the null distribution of the χ^2 statistic — i.e., the distribution of χ^2 which results when H_0 (Poisonness) is true.
1st (25-count) Filter			
3	1	1.04	69%
4	2	3.32	81%
5	3	1.60	34%
6	4	2.60	37%
7	5	3.84	43%
2nd (38-count) Filter			
3	1	0.21	35%
4	2	4.32	88%
5	3	2.79	57%
6	4	3.68	55%
7	5	6.21	71%

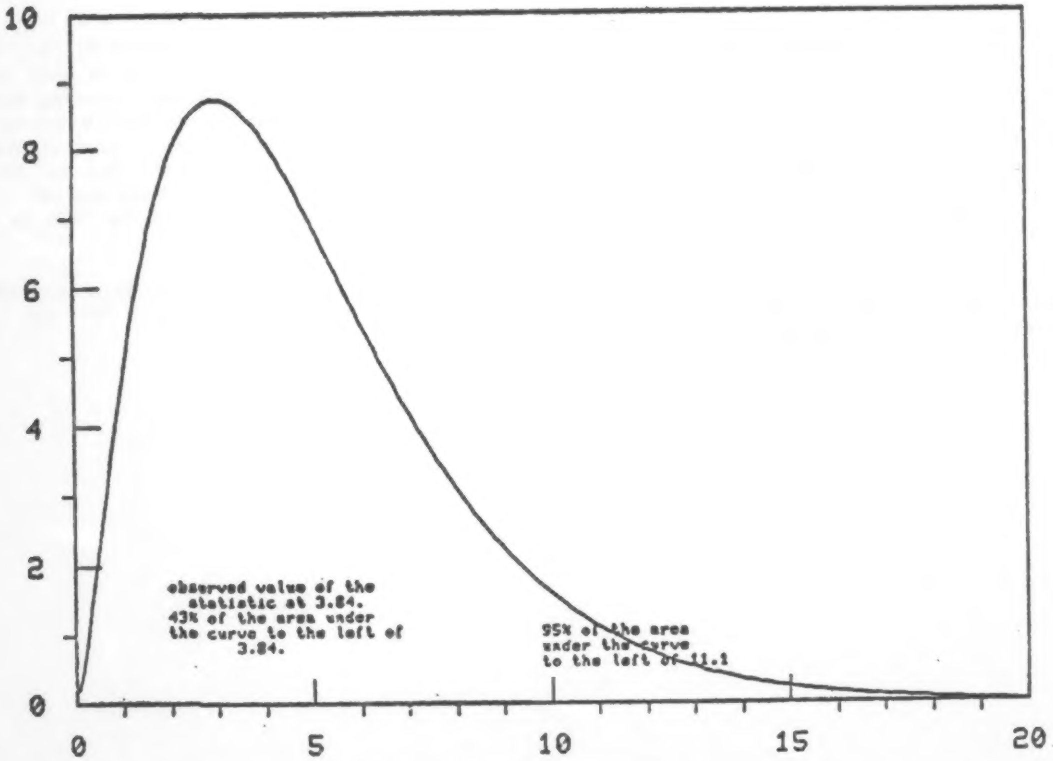

Figure 9. Chi-square probability density function for five degrees of freedom. (1st filter, 7-partition case)

Table 3. Kolmogorov-Smirnov Test.

$D = \sup \; S(x) - P(x)$ S = empirical CDF P = Poisson CDF	Percent point value of observed D. The percent point value is from the null distribution of the D statistic – i.e., the distribution of D which results when H_0 (Poisonness) is true.
For 1st (25-count) Filter	
0.065	20%
For 2nd (38-count) Filter	
0.158	70%

6. Filter Homogeneity

An important characteristic of both reference filter standards and field analysis filters is homogeneity. Intuitively, the homogeneity of a filter refers to randomness of scatter plus uniformity, where we can define uniformity as meaning that the projections of the counts occurring on the grid onto the x and y axes are near uniform.

Two checks of homogeneity were performed. The first was done specifically to see if there was a discernible radial gradient in the filter density. A quick visual test of the existence of such a gradient was performed by subtracting off the mean count value $(\bar{x})$ for the entire filter from each of the individual grid square counts, and coding the resulting residuals with respect to a size comparison with the standard deviation for the counts over the entire filter: positive residual less than one standard deviation are coded "+", negative residuals less than one standard deviation are coded "-", positive residuals greater than one standard deviation are coded "++", and negative residuals greater than one standard deviation are coded "--". Figure 10 depicts these coded residuals over the surface of the second uniform filter. Aside from a higher density patch in the northwest corner of the figure, no consistent radial density gradient is evident. One possible explanation of the high density patch might be a slight tilt to the filter during the preparation procedure.

A second test of homogeneity was done by partitioning the set of counts from the second filter in various ways to mimic physically slicing the filter as shown in figure 10. Then the means and variances of the distributions of counts resulting on the various slices were compared using a t-test (not assuming equal variance) for the means and F-test for the variances. The results are shown in Table 4. The mean counts and variances are comparable in almost all cases at 5 percent significance. Even for partition number 3, where one might expect a difference in the mean loadings to be detected from the residual plot shown in figure 10, no difference was seen. Another method of analysis would have been to do an ANOVA (F-test) on each partition. Generally, the "all possible t-tests" approach leads to more significant differences than the F-test approach.

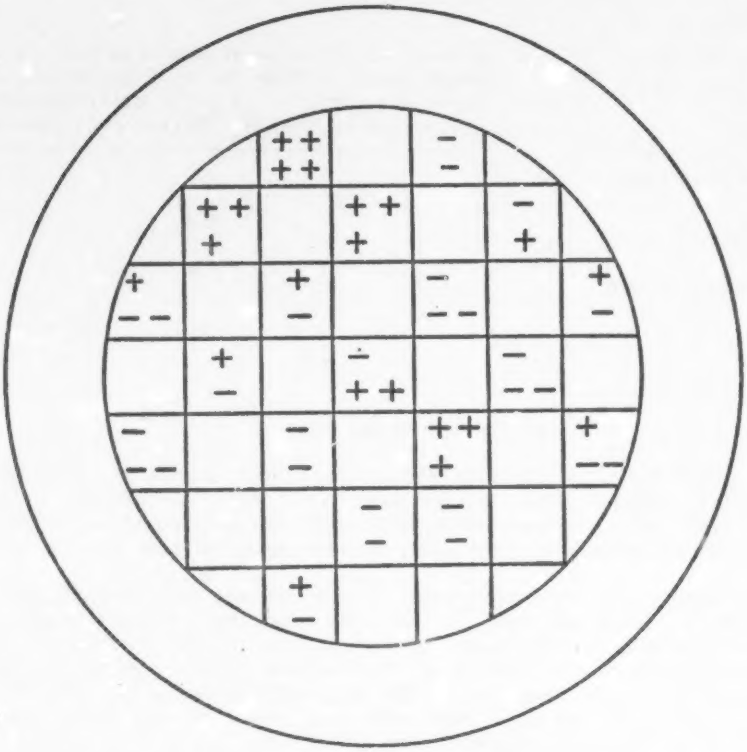

Figure 10. Schematic of the coded residuals for the second uniform filter.

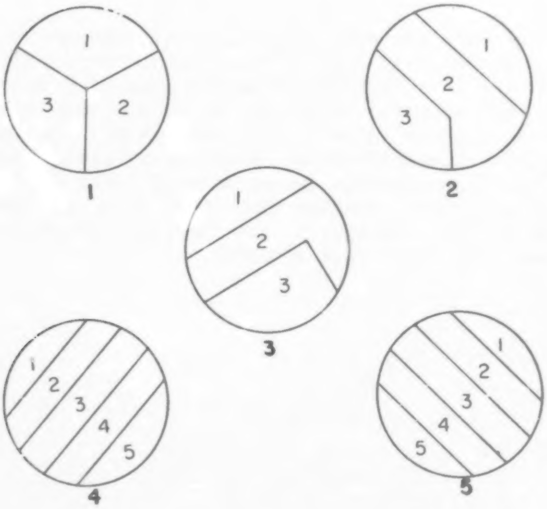

Figure 11. Uniform flter partitionings.

Table 4. Results of t- and F-Test.

Partition	Comparing	Means Comparable	Variances Comparable	Means				
				1	2	3	4	5
1	1-2	Y	Y	26	24			
	1-3	NO	Y	26		23		
	2-3	Y	Y		24	23		
2	1-2	Y	Y	25	26			
	1-3	Y	Y	25		23		
	2-3	NO	Y		26	23		
3	1-2	Y	Y	25	25			
	1-3	Y	Y	25		24		
	2-3	Y	Y		25	24		
4	1-2	Y	Y	28	24			
	1-3	Y	Y	28		24		
	1-4	Y	Y	28			25	
	1-5	Y	Y	28				22
	2-3	Y	Y		24	24		
	2-4	Y	Y		24		25	
	2-5	Y	Y		24			22
	3-4	Y	Y			24	25	
	3-5	Y	Y			24		22
	4-5	Y	Y				25	22
5	1-2	marginal	marginal	25	25			
	1-3	Y	Y	25		27		
	1-4	Y	Y	25			23	
	1-5	Y	Y	25				21
	2-3	Y	Y		25	27		
	2-4	Y	Y		25		23	
	2-5	Y	Y		25			21
	3-4	NO	Y			27	23	
	3-5	NO	NO			27		21
	4-5	Y	Y				23	21

7. Conclusions

There have been several discussions of distributions of fiber counts on filters [10-13], relating the merits of various assumptions concerning the underlying distribution of counts.

This paper shows that at least for liquid filtration of chrysotile onto 0.1 and 0.4 μm Nuclepore filters with fiber loading densities in the range of 10-70 fibers per 200 mesh grid square, one may expect a Poisson distribution. This hypothesis can and should be tested for every filter. Once the tests for Poissonness have been passed, arbitrarily precise loading estimates can be established using the above methods and reported with the filter analysis.

A carefully characterized filter has been shown to be homogeneous on a level that allows sections of the filter to be considered representative of the whole filter. The next step of this research is to use such well-characterized filters to carry out split sample round robins with experienced laboratories to better characterize instrument and operator effects on asbestos analysis.

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REFINEMENTS IN THE EPA PROVISIONAL METHODOLOGY

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Abstract

Although the electron microscopical method is the best available to assess the asbestos minerals in the environment, the credibility of the analytical technique is weakened due to variations in the methodology with resulting diversity of data. The EPA Provisional Methodology on the electron microscope measurement of airborne asbestos concentrations was developed from a study under EPA Contract No. 68-02-2251 to evaluate the various electron microscopic methods to arrive at an optimum composite procedure. The lack of properly defined standard procedures restricted its acceptance as the standard analytical technique.

The present study under Contract No. 68-02-3266 investigated the problem areas of: (1) sample collection; (2) sample transport; (3) sample preparation procedures; (4) identification of particles as fibers; and (5) verification of asbestos materials. The critical parameters of which filter type (polycarbonate or cellulose ester type), transfer method, and fiber identification were also examined.

The expertise of the particle technologist and the electron microscopist have been combined to refine the EPA Provisional Methodology to obtain a more consistent analysis. Three levels of analytical sophistication are advocated.

1. Introduction

The EPA Provisional Methodology was first published in August, 1977. A revised edition dated June 1978 was subsequently distributed. The methodology based on the electron microscope measurement of airborne asbestos concentration was developed under EPA Contract No. 68-02-2251. Various electron microscopic methods were evaluated to arrive at an optimum composite procedure. The lack of properly defined standard procedures and collaborative testing of prepared samples restricted its acceptance as a standard analytical technique.

Under Contract No. 68-02-3266, the Provisional Methodology is being evaluated relative to (1) sample collection, (2) sample transport, (3) sample preparation procedures, (4) identification of particles as fibers, and (5) verification of asbestos materials. Specifically some of the parameters requiring study were:

1) the recommendation of polycarbonate filters;
2) the acceptance of high volume filtration;
3) the effects of sampler face velocity;

4) the loss of fibers from handling and transport;
5) the role of fiber bundles, and
6) the role of energy dispersive x-ray analysis.

The information being presented are preliminary results of the present study.

2. Purpose and Use of the Methodology

We have found that one of the major reasons for the variability in results and consequently the acceptance of the methodology was the misunderstanding of the purpose as well as the misuse of it. The usage of electron microscopy (EM) in asbestos analysis is tedious, time-consuming, and expensive. The determination of asbestos in air, water, food, and miscellaneous bulk materials would require a tremendous number of analyses. No one method would provide all the answers such as number concentrations, mass concentration, length/width size distribution, asbestos or non-asbestos, kind of asbestos, etc.

The Provisional Methodology was designed to provide as many answers as possible, to be relatively fast, and to be economical – essentially a screening tool to search, recognize, and pinpoint the presence of asbestos fibers in the air. The method would provide the number, an unaltered size distribution, a calculated mass derived from it, and information whether it was chrysotile, amphibole, or not. The method is not for use to determine mass concentration in air or in a bulk sample nor to provide a classification of amphibole asbestos species (crocidolite, amosite, tremolite, anthophyllite, or actinolite), nor to go to court to cite a facility. The method, however, can be used for airborne asbestos from source emissions, but only within the constraints or limitations of the methodology.

The use of selected area electron diffraction (SAED) from a visual analytical scheme is recognized as a subjective method for rapid screening. Emphasis here is not on the possibility of misidentification but to err on the side of health and safety. A "false" positive can lead to a recheck or repeat analysis whereas a "false" negative may lead to complacency.

Knowing its limitations, if the method is used for its intended purpose, a great deal of uncertainty will be eliminated. However, for classification and to place the methodology in its proper perspective, we have classified asbestos analysis into three levels of effort; each level requires a greater degree of expertise, training, time and cost.

A Level I analysis would be the Provisional Methodology requiring an air sample, sample preparation, and a TEM for obtaining a number count, size distribution, visual SAED analysis, and a derived mass concentration. A Level II analysis will be the Provisional Methodology plus energy dispersive x-ray fluorescence spectra analysis. A Level III analysis would be a TEM analysis plus energy dispersive x-ray spectra analysis plus quantitative SAED analysis. A Level III analysis will stress identification of asbestos species with less stress on the number and size representation in the sample. The Level III analysis will be used most probably with bulk samples and source samples and these will require special precautions relative to sampling, contamination, counting, etc. In reality, polarized light microscopy and x-ray diffraction analysis will probably be used prior to electron microscopy. A combination of levels is possible for special situations.

Placed in its proper perspective, the following discussion is with the Provisional Methodology and its goal of providing reproducible number, length, width, kind of mineral (chrysotile, amphibole, or not), and a calculated mass. The emphasis is to eliminate or minimize all parameters that effect reproducibility or the usage of a technique that can be performed by only a limited few. As a monitoring tool, the methodology must be available to all laboratories.

3. Sample Collection

8" x 10", 102 mm, and 47 mm samplers were evaluated. The 8" x 10" samplers were tested out in the field. The 102 mm and 47 mm samplers were tested in an 8' x 8' x 8' aerosol box. Field personnel have difficulty in handling 102 mm and the 8" x 10" filters.

Use of a filter cassette for the 8" x 10" minimizes one of the problems for the field worker, but creates problems for the laboratory personnel. The basic problems are the fragility of cellulose ester filters and the flexibility of the polycarbonate filters. Reproducible handling of 8" x 10" and 102 mm filters was impossible in the field, especially during inclement weather. We do not recommend the use of these larger filter sizes for field sampling. For sample collection and transport the cellulose ester type filters collected the aerosol uniformly and the filter could be transported to the lab with a minimum of loss. Polycarbonate filters also collected the aerosol uniformly. However, retention of particulates, especially the larger sizes, were very poor. Based on x-ray fluorescence data, approximately 40 percent of the mass was lost in transit. Based on these results, cellulose ester type filters would be selected for sampling. However, for very small particulates, shipping the polycarbonate filters through the mail resulted in negligible loss based on XRF data.

4. Effects of Sampling Velocities

The 102 mm and 47 mm filters were selected to study the effect of face velocity on the collection efficiency of polycarbonate *vs*. cellulose ester type filters. The tests were conducted in the 8' x 8' x 8' aerosol box using chrysotile aerosol. The sampling heads were attached to a probe with the vacuum pumps outside the box. The uniformity of aerosol and sampling head position were established prior to conducting face velocity tests. Collection efficiency was determined by x-ray fluorescence analysis of the filter surface. Efforts were made to collect only the fines of the aerosol by delaying sampling for four hours after generation. The sampling times were carefully selected to obtain a very light loading of chrysotile on the filter – not to exceed a mono-layer. The emphasis was on the filter's efficiency and not on the asbestos fiber filtration efficiency. Face velocities of 3.0, 9.0, 21.2, 28.8, and 34.8 cm per second were tested. At each face velocity, both types of filters were tested. Lastly, each type of filter substrate was tested at three different face velocities. Preliminary results show that for these aerosols, capture efficiency was independent of face velocity. No noticeable difference was found between the polycarbonate and the cellulose ester. The 47 mm filters appeared to have a higher collection efficiency than the 102 mm filters. This may be due to the mounting technique used for the XRF. In the 102 mm filter a circular 47 mm diameter section is cut for mounting in the XRF cassette, whereas the 47 mm filter required no cutting. For ease in handling, costs, and filtration advantage, the 47 mm filter is recommended for aerosol sampling.

The use of a sampling system designed for the analysis of Total Suspended Particulates (TSP) levels for use in asbestos analysis by the substitution of filter substrates is one of the causes for the present variability in results. TSP analysis uses high volume sampling for 24 hours using glass fiber filters. High volume is used to grab enough air sample to obtain a weighable fraction, 24 hours to average fluctuations in particulate loadings from day and night activities, and glass fibers to provide minimum pressure drop with maximum collection of solids, liquids, and reaction products.

For asbestos analysis, a sampling system called "hydra" has been designed and is presently being constructed for testing. The system is a set of individual samplers mounted in a multi-headed arrangement and utilizing different sampling face velocities for a constant sampling period. The sampling period is adjusted to accomodate a *minimum* of three (3) 47 mm filters for particulate loadings (low, medium, high), a 47 mm filter for weighing and XRF anslysis, a sampler position for a field blank, plus additional sampling ports to accomodate a midget impinger as well as a cascade impactor.

Total suspended particulates range from a low of 10 μg/m^3 (remote, non-urban) to 60 μg/m^3 for near urban to 220 μg/m^3 for urban areas. However, for heavily polluted areas, TSP levels up to 2000 μg/m^3 may be possible. From the electron microscopist's viewpoint, a loading of 5-10 μg/cm^2 of filter is adequate for analysis. Values beyond 20-25 μg/cm^2 will require a dilution treatment. For a 47 mm filter at a face velocity of 3 cm/sec (2.48 ℓ/min), 9.0 cm/sec (7.45 ℓ/min), and 21.2 cm/sec (17.62 ℓ/min) in 30 minutes, 74.4, 223.5, and 528.6 liters of air, respectively are sampled. For an urban area of 200 μg/m^3, 14.88 μg, 44.7 μg, and 105.7 μg would be collected on the 47 mm filter (eff. filtration area of 13.85 cm^2), or 1.07 μg/cm^2, 3.23 μg/cm^2, and 7.63 μg/cm^2. The sampling time could be increased to 60 minutes for a non-urban area or reduced in a heavily polluted area (source emissions).

The system advantages are:

1) It is less costly in time, effort, equipment, and materials.
2) The sample preparation steps are minimized and can be related to TSP levels.
3) The complementary analysis of field blank, TSP, bacteria, size distribution, etc., is possible.
4) It can accomodate ambient air sample and source emission samples.

The system disadvantages are:

1) A short sampling period may catch an episode.
2) The sampling quantity on volume is small and may not indicate the presence of asbestos fibers.

On completion of the assembly, the sampling system will be tested. The detection limit for the 47 mm filter sampling 1 m^3 of air is 2×10^4 fibers/m^3.

5. Sample Preparation

5.1 Jaffe Wick Washer

Each user of the Jaffe Wick Washer makes slight modifications to the basic methodology. Some of the modifications require greater dexterity, while others require greater attention. Figure 1 is a schematic of the modified Jaffe Wick Washer. The following changes were made:

1) The petri dish cover and bottom were ground to obtain a closer fit and minimize refilling the petri dish with added solvent. We find that the substrate should be kept constantly wet for continuous wicking action to occur. Total time for the dissolution of the filter may take up to 40 hours.

2) The foam substrate or the use of a stack of 5 1/2 cm filter paper had small problems. A combination of the foam plus a single sheet of 5 1/2 cm filter paper provided an economical disposable substrate for the Jaffe Wick Washer.

3) The EM grid-polymer sandwich resting on the stainless steel mesh screen should be picked up by the mesh screen while wet on completion of the polymer solution and set on lens paper tacked to the bottom of a separate petri dish. The grid is then lifted from the screen to dry adjacent to the screen.

4) The use of the micro-drop of solvent to tack the filter material to the EM grid resulted in a high percentage of curled and ruined EM grid-filter sandwiches. We recommend holding the sandwich at the edges with the tweezers and laying it directly on a damp 100 mesh screen.

5.2 Filter Selection

In the selection of a filter for transfer, the polycarbonate filters have been accepted as the substrate providing minimum loss during transfer to an electron microscope grid using the modified Jaffe Wick Washer. There is a great loss of particulates in transferring from cellulose ester filters using the modified Jaffe Wick Washer. The loss is reduced significantly if the cellulose ester is first carbon-coated as with the polycarbonate type. However, the analyst has a difficult time seeing fibers in the irregular replica surface especially of the small fibers. Reproducibility of the results depends greatly on the individual EM operator. The poor transfer technique capability of cellulose ester filters appears due to the thickness of the filter, which is 150 μm vs. 10 μm for Nuclepore.

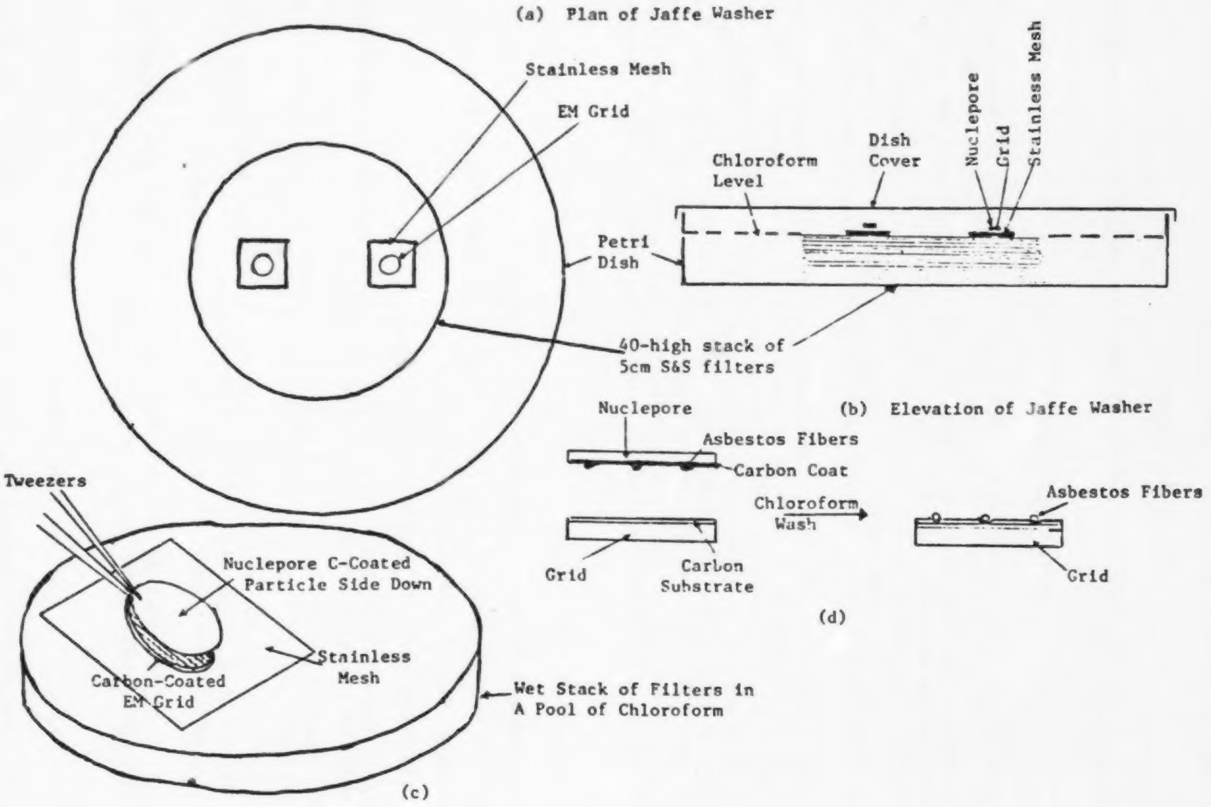
(a) Plan of Jaffe Washer
Stainless Mesh
EM Grid
Petri Dish
40-high stack of 5cm S&S filters
Chloroform Level
Dish Cover
Nuclepore
Grid
Stainless Mesh
(b) Elevation of Jaffe Washer
Nuclepore
Asbestos Fibers
Carbon Coat
Grid
Carbon Substrate
Chloroform Wash
Asbestos Fibers
Grid
(d)
Tweezers
Nuclepore C-Coated Particle Side Down
Stainless Mesh
Carbon-Coated EM Grid
Wet Stack of Filters in A Pool of Chloroform
(c)

Figure 1. Modified Jaffe Washer Method.

(a) Plan view
(b) Elevation view of Jaffe washer
(c) Details for placing a specimen for washing
(d) Principle of the Jaffe method

At present, we are investigating the fused membrane technique utilized by NIOSH. Criticism of the NIOSH technique is the possible loss of small fibers in the fused layer.

Presently, we have no well-defined method of utilizing cellulose ester type filters. Ashing as practiced successfully in some labs does not give the same consistency when done by others. The methodology must be refined for others to duplicate the same success reported by Dr. Chatfield at this meeting. If the sampling system called "Hydra" is successful, we may not need to use cellulose ester type filters.

5.3 Gold Coating

The prepared grid is gold-coated very thinly in order to establish an internal standard for selected area diffraction analysis.

5.4 Fiber Identification

During participation in interlaboratory testing of fiber counting, both optical and electron microscopy, we find that counting discrepancies are minimized if more explicit counting rules are made. The decision to count or not to count can be simplified by instructions supplemented by sketches. In the Provisional Methodology, the term "fiber" is used for a particle with an aspect ratio of 3:1 or greater with substantially parallel sides. Using this definition, edge views of flakes, fragments from cleavage planes, scrolls, etc., may be counted as fibers. A consistency in counting is the desired goal.

Unfortunately, all fibers do not stand isolated to be counted. Fibers are with other particulates in varying arrangements as well as to each other. We now characterize asbestos structures wherein fiber is one category. The others are bundles (B), clusters (Cl), and matrices (M), by themselves or in combination with each other. Bundles are fibers in a parallel arrangement with each fiber closer than one fiber diameter. Clusters are fibers in a random arrangement such that all the fibers are intermixed and no single fiber is isolated from the group. Matrix is a fiber or fibers with one end free and the other end is embedded or hidden by a particulate. Combinations can be a matrix and cluster (M-Cl), bundle and a matrix (B-M), etc.

Counting rules for single fibers are:

1) Particulates meeting definition of fiber – isolated by itself.
2) Count as single entities, if separation is equal or greater than 1 diameter.
3) Count as single entities, if 3 ends can be seen.
4) Count as single entities, if 4 ends can be distinguished.
5) Two or more fibers are counted as a bundle if the distances between fibers are less than one diameter or the ends cannot be resolved.
6) Fibrils attached longitudinally to fibers are counted as part of the fiber and size estimated (width) based on fiber to fibril relationship.
7) Fiber partially hidden by grid wires is counted if it is on the top or right edge and not counted if on the left or bottom edge. Here the choice is to be consistent and to use two of the four sides of the grid opening.

At this meeting, counting rules may be adopted that may reflect the concensus of the group. We are open to suggestions, corrections, and criticisms.

6. Conclusion

This paper is not the complete changes, modifications, or deletions in the EPA Provisional Methodology. We are still working on problem areas in order to obtain more consistent results in asbestos analysis. We appreciate any and all help that we can get.

A sample data sheet for the EM operator is shown in figure 2 for guidance. Asbestos structures will have a very crude estimate of size.

Sample No. ____________ Acc. Voltage ____________ Date ____________

Filter Type ____________ Beam Current ____________ Grid Box ____________

Filter Area ____________ Magnification ____________ Grid Location ____________

Comments ____________ Grid Area ____________ Analyst ____________

CO	Fiber #	Struct.	Dimension		SAED Observation					SAED/Image		EDS	ID
			Width	Length	CHRYS	AMPH	AMBIG	NON-A	NO-P	0°	40°		

Figure 2. Asbestos analysis record.

National Bureau of Standards Special Publication 619. Proceedings of the NBS/EPA Asbestos Standards Workshop held at NBS, Gaithersburg, Maryland, October 1-3, 1980. (Issued March 1982)

FIBER IDENTIFICATION AND BLANK CONTAMINATION PROBLEMS IN THE EPA PROVISIONAL METHOD FOR ASBESTOS ANALYSIS

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Abstract

Two problems with the EPA provisional method for analysis of airborne asbestos by electron microscopy are identified. These problems are 1) an inadequate discussion of blank contamination problems and 2) fiber identification procedures that are not rigorous enough to prevent some minerals from being misidentified as asbestos. In order to illustrate the blank problem, the additive nature of chrysotile fiber contamination in the three stages of a cellulose acetate blank filter preparation procedure is demonstrated. Increases in fiber lengths and widths as well as numbers in successive preparation stages are shown to be statistically significant. Considerable variability was found in fiber numbers from filter to filter at each preparation stage. Metropolitan air sample chrysotile concentration and size data are compared with blank concentration and size data. The potential for confusing amphibole diffraction patterns with other minerals (including palygorskite, sepiolite, enstatite, hypersthene, magnetite, laumontite and minnesotaite) is discussed. A more rigorous procedure for identifying asbestos minerals, incorporating both energy dispersive x-ray analysis and tilting the fibers to obtain zone axis diffraction patterns is recommended.

The EPA's efforts to develop standard procedures for analysis of asbestos by electron microscopy have vastly improved the quality of those analyses. They correctly emphasized the development of standard techniques of sample preparation and analysis protocol. Until this standardization occurred, analysis of the same sample could vary by a factor of 1000 from lab to lab. Under these circumstances, errors due to fiber identification or contamination problems were minor compared to errors resulting from preparation and counting procedures. At the present time, however, interlaboratory agreement on the same sample has progressed to the point where errors other than those involved with sample preparation are important. Despite the fact that several sample collection and preparation problems remain, a standard method for analyzing asbestos in air should include a thorough discussion of other sources of error in the technique.

In our use of the current EPA provisional method, we have found several deficiencies. In this paper, we will discuss two of them and offer recommendations for improvement.

1. There is an inadequate discussion of asbestos contamination during sample preparation, particularly with the ashing alterative. The method should require that effective blank levels be reported with fiber concentrations and some means of determining whether or not sample concentrations are significantly above blank level could also be included.

2. The fiber classification procedures are not rigorous enough to prevent other materials from being classified as asbestos. The method should provide for energy dispersive x-ray spectroscopy (EDS) and indexing of electron diffraction patterns to prove the presence of amphibole minerals. Then, if there are no significant interfering minerals present, less rigorous techniques may be used.

1. Blank Contamination

Chrysotile fibers are often found as a contaminant on unused filters and ashing dishes. The provisional method notes that contamination might be a problem but does not discuss the possible variability in blank levels nor does it recommend that blank concentrations be reported. In order to illustrate these problems and show how important they are to the interpretation of some air samples, we will give the results of a study of the numbers and sizes of chrysotile fibers in blanks and air samples.

The components of the sample preparation procedure were examined to determine the blank levels of chrysotile. This attempt was complicated by the extreme variability in chrysotile contamination levels found in the blank filters and in the ashing procedure used to eliminate the cellulose acetate filter material. The large standard deviation values for the concentration of fibers in the Millipore and Nuclepore original filters and the ashed dish Nuclepore filters listed in Table 1 illustrate this variability. Fiber concentrations for a given type of filter often varied by a factor of 100 or more.

Table 1. Mean Fibers/mm^2 and their Standard Deviations for Millipore Filters, Nuclepore Filters and Ashed Empty Dishes.

Sample Composite	No. Samples	No. Fibers	Original Filter $\bar{x}$ Fibers/mm^2	$S_{\bar{x}}$
CHRYSOTILE				
0.1 μm pore size, 47 mm dia. Nuclepore	26	219	97.76	149.63
Ashed empty dish on 0.1 μm, 47 mm Nuclepore	31	731	466.10	870.71
0.22 μm pore size, 47 mm dia. Millipore	6	205	340.68	431.85
0.8 μm, 102 mm dia. Millipore	20	641	861.02	2224.90

Our blank preparation consisted of three main components. These were:

1. The cellulose acetate (Millipore) filter.
2. The ashing dish in which the Millipore filter was oxidized.
3. The polycarbonate (Nuclepore) filter onto which the resuspended ashed Millipore was filtered.

Chrysotile contamination from these three components was additive and only the blank Nuclepore filter could be examined apart from the other components. All of the filters and the ashing dish were counted on Nuclepore filters.

The median ($\tilde{x}$) concentration (fibers per square millimeter) on the original filter and standard error of the median ($SE_{\tilde{x}}$) values for two types of ashed Millipore filter and the Nuclepore filter were calculated, as well as the median concentration on the grid for the ashed empty dish Nuclepore and the ashed 0.8 μm pore size, 102 mm diameter Millipore. Table 2 is a list of these values. Median values were used as an estimate of the fiber levels on the filters since they were not drastically affected by the extreme values encountered.

Table 2. $\tilde{x}$ Fibers/mm^2 and $SE_{\tilde{x}}$ for the Blank Filters and Ashing Dish and $\tilde{x}$ Fibers/Grid Opening and $SE_{\tilde{x}}$ for the Ashed Empty Dish the 102 mm Diameter Millipore, and the 47 mm Nuclepore.

Sample Composite	$\tilde{x}$ Fibers/mm^2 (On Original Filter)	$SE_{\tilde{x}}$ Fibers/mm^2 (On Original Filter)	$\tilde{x}$ Fibers/mm^2 (On the Grid)	$SE_{\tilde{x}}$ Fibers/mm^2 (On the Grid)
47 mm Nuclepore	41.05	37.14	41.05	37.14
Ashed Dish	138.00	195.48	138.00	195.48
102 mm Millipore	164.50	621.88	324.00	234.95
47 mm Millipore	136.00	220.38		

Using a z score test [1][1], the median concentrations of fibers on the grid for the various filters and the ashed dish were compared, except for the two Millipores where the concentrations on the original filter were used. Table 3 is a list of these z score values, their corresponding p values, and the conclusions drawn from these values. The z score was used to test the hypothesis (null hypothesis) that there was no difference in the fiber concentrations on the ashed Millipore and blank Nuclepore filters and on the ashing dish treated Nuclepore. It was found that the median fiber level values were significantly different for all of the preparations compared except for the two types of Millipore. Each step in the preparation process apparently added fibers to the total count found on the ashed Millipore. Both types of Millipore filter contained about the same concentrations of fibers.

Table 3. Comparison of Median Chrysotile Concentration Values for the Millipore and Nuclepore Filters and the Ashed Dish.

Comparison	z Score	P (One Tail)	Conclusion (0.01 Level)
Ashed Dish/Nuclepore	2.70	0.00345	Reject H_o
102 mm Millipore/Ashed Dish	2.94	0.00170	Reject H_o
102 mm Millipore/47 mm Millipore	0.17	0.43250	Fail to Reject H_o

In order to further assess the possible differences in the chrysotile contamination encountered at various stages in the sample preparation procedure, the size distribution of the fibers found at each stage were examined. Figure 1 is a set of graphs of the percentage cumulative distributions for fibers found in each of 12 length categories for the Nuclepore, 0.8 µm, 102 mm diameter Millipore, 0.22 µm, 47 mm diameter Millipore and the ashed empty dish composite samples. Figure 2 is a set of graphs of the cumulative percentage distributions for the 11 width categories. A comparison of these graphs indicated a trend toward increasing numbers of fibers in the larger size categories for both length and width as the ashing dish and Millipore components were added to the Nuclepore. Using the Kolmogorov-Smirnov two sample test for determining significant differences in cumulative percentage distributions [2], it was found that the apparent length and width differences were significant. Table 4 is a summary of the statistical findings and a list of the mean and median fiber widths and lengths. In comparing the width distributions of the ashed empty dish and the 0.22 µm, 47 mm diameter Millipore, it was found that significant differences could not be demonstrated. The length distributions, however, were significantly different. The comparisons of the 47 mm and 102 mm Millipores showed no significant length or width differences. A one tailed Kolmogorov-Smirnov two sample test was used in all cases since it was reasonable to hypothesize an increase in fiber lengths and widths from one blank component to the next, based on the appearance of the cumulative percentage distributions.

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[1] Figures in brackets refer to the literature references at the end of this paper.

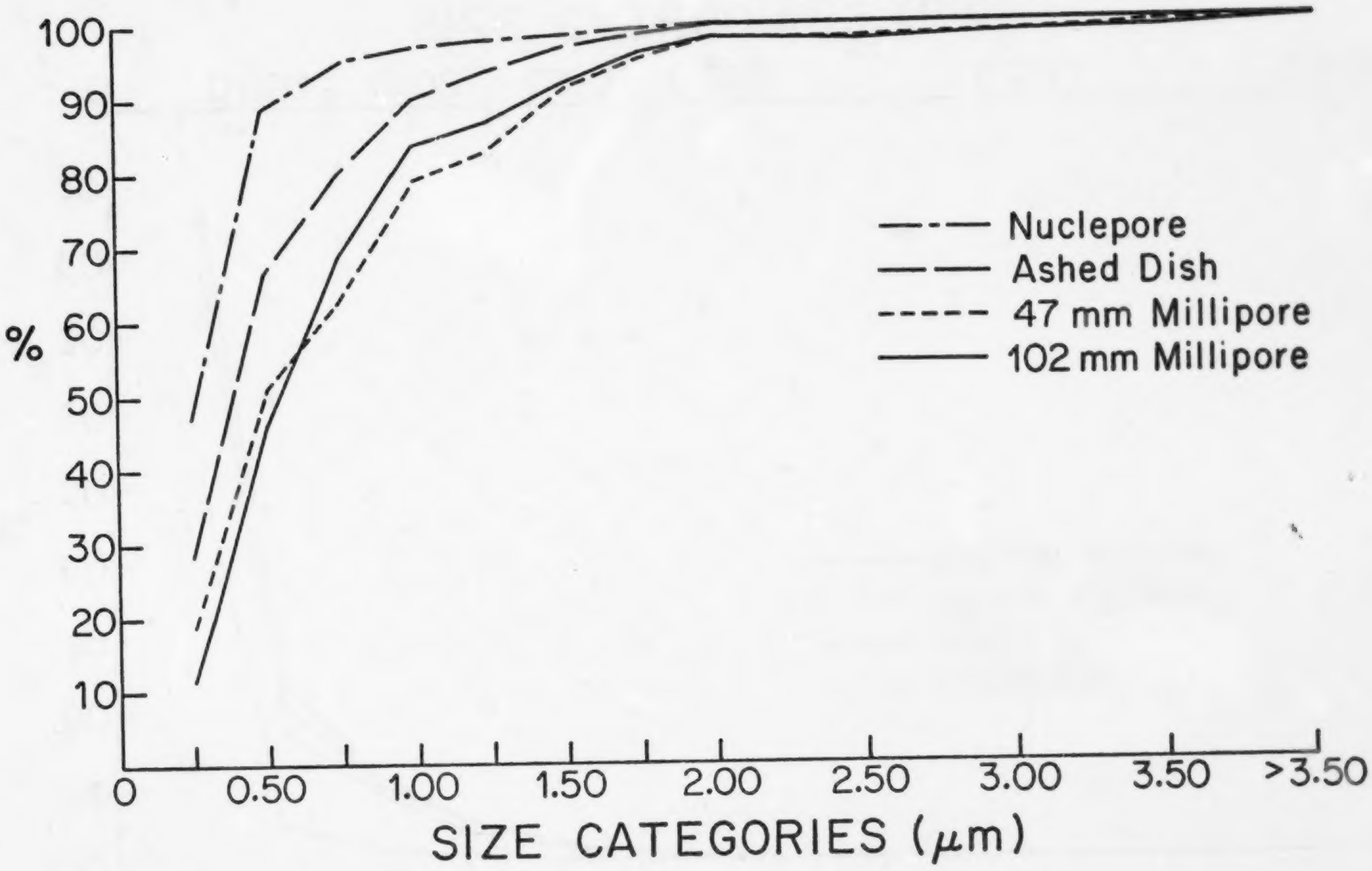

Figure 1. Cumulative percentage distributions for chrysotile lengths in blanks.

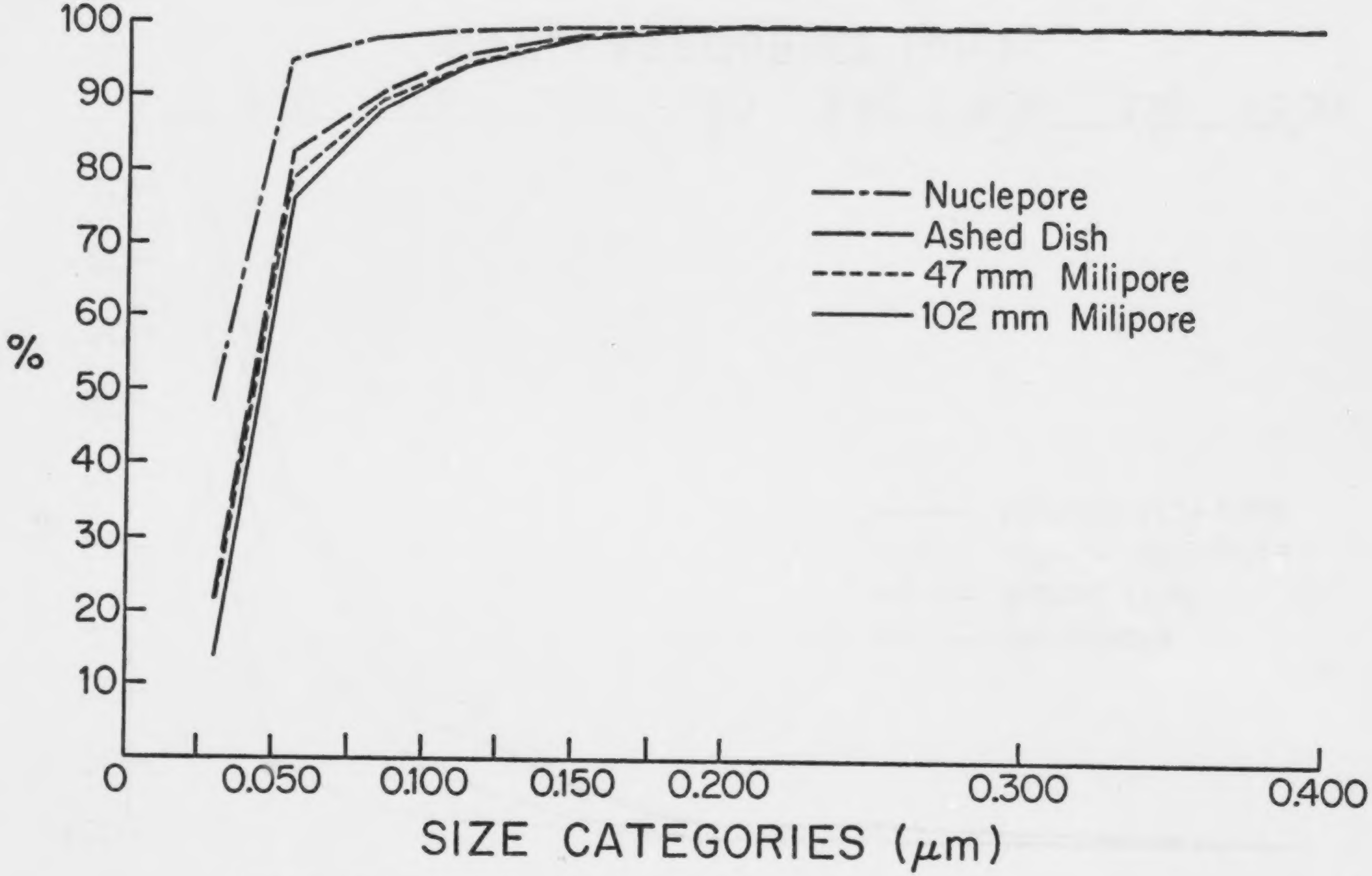

Figure 2. Cumulative percentage distributions for chrysotile widths in blanks.

Table 4. Kolmogorov-Smirnov Two Sample Test Results for Significant Differences for Chrysotile Fiber Widths and Length Cumulative Percentage Distributions and a List of Fiber Width and Length Central Values.

Comparison	Dimension	χ^2	Conclusion (0.01 Level)
Nuclepore/Ashing Dish	Width	42.60	Reject H_o
Nuclepore/Ashing Dish	Length	31.71	Reject H_o
47 mm Millipore/Ashing Dish	Width	0.60	Fail to Reject H_o
47 mm Millipore/Ashing Dish	Length	18.73	Reject H_o
102 mm Millipore/Ashing Dish	Width	13.23	Reject H_o
102 mm Millipore/Ashing Dish	Length	57.75	Reject H_o
47 mm Millipore/0.8 Millipore	Width	4.52	Fail to Reject H_o
47 mm Millipore/0.8 Millipore	Length	7.10	Fail to Reject H_o
47 mm Millipore/C.C. Nuclepore	Width	29.63	Reject H_o
47 mm Millipore/C.C. Nuclepore	Length	60.71	Reject H_o
102 mm Millipore/C.C. Nuclepore	Width	79.89	Reject H_o
102 mm Millipore/C.C. Nuclepore	Length	17.75	Reject H_o

Samples	$\bar{x}$ Width (μm)	$\tilde{x}$ Width (μm)	$\bar{x}$ Length (μm)	$\tilde{x}$ Length (μm)
Nuclepore	0.0350	0.033	0.335	0.280
Ashed Dish	0.0490	0.042	0.511	0.370
47 mm Millipore	0.050	0.042	0.728	0.480
102 mm Millipore	0.053	0.042	0.755	0.550

After determining the median blank chrysotile levels for ashed Millipore filters, an examination of ashed air samples collected on 0.8 μm pore size, 102 mm diameter Millipore filters was undertaken. Seven ashed air sample chrysotile counts were obtained and mean, standard deviation, median, and standard error values were calculated. Table 5 is a summary of these findings. The z score test [1] was done to test the hypothesis that there was no difference in the median fiber concentrations between the ashed air samples and the ashed Millipore blanks. A significant difference was found. More chrysotile fibers were present on this particular set of air samples than were found on the Millipore blanks. The z score results are listed in Table 6.

Cumulative percentage distributions for the widths and lengths of the total fibers on the air samples and the 102 diameter Millipore are presented in figures 3 and 4. The apparent tendency toward wider and longer fibers in the air samples was tested using the Kolmogorov-Smirnov test. The results, summarized in Table 7, show that significant differences in these size distributions do exist.

Table 5. Metropolitan Air Sample Data for One Collection Site.

Sample	Fibers/Cu. Meter	Fibers/mm^2
1	9.92×10^3	844.96
2	1.59×10^4	1369.98
3	8.94×10^3	502.50
4	6.04×10^3	1850.00
5	1.51×10^4	1960.00
6	1.03×10^4	1270.00
7	1.09×10^4	4230.00

$\bar{x}$ Fibers/mm^2 (Original Filter) = 1718.20

$S_{\bar{x}}$ Fibers/mm^2 = 1221.17

$\tilde{x}$ Fibers/mm^2 (Original Filter) = 1369.98

$SE_{\tilde{x}}$ Fibers/mm^2 = 576.95

Table 6. z Score Results for the Comparison of the Metropolitan Air Sample and 0.8 µm, 102 mm Millipore Fibers/mm^2 Median Values.

Comparison	z Score	P (One Tail)	Conclusion (0.1 Level)
Air Sample/0.8 µm Millipore (Fibers/mm^2)	4.66	0	Reject H_0

Median Values

0.8 µm, 102 Millipore x Fibers/mm^2 = 861.02

Air Sample x Fibers/mm^2 = 1369.98

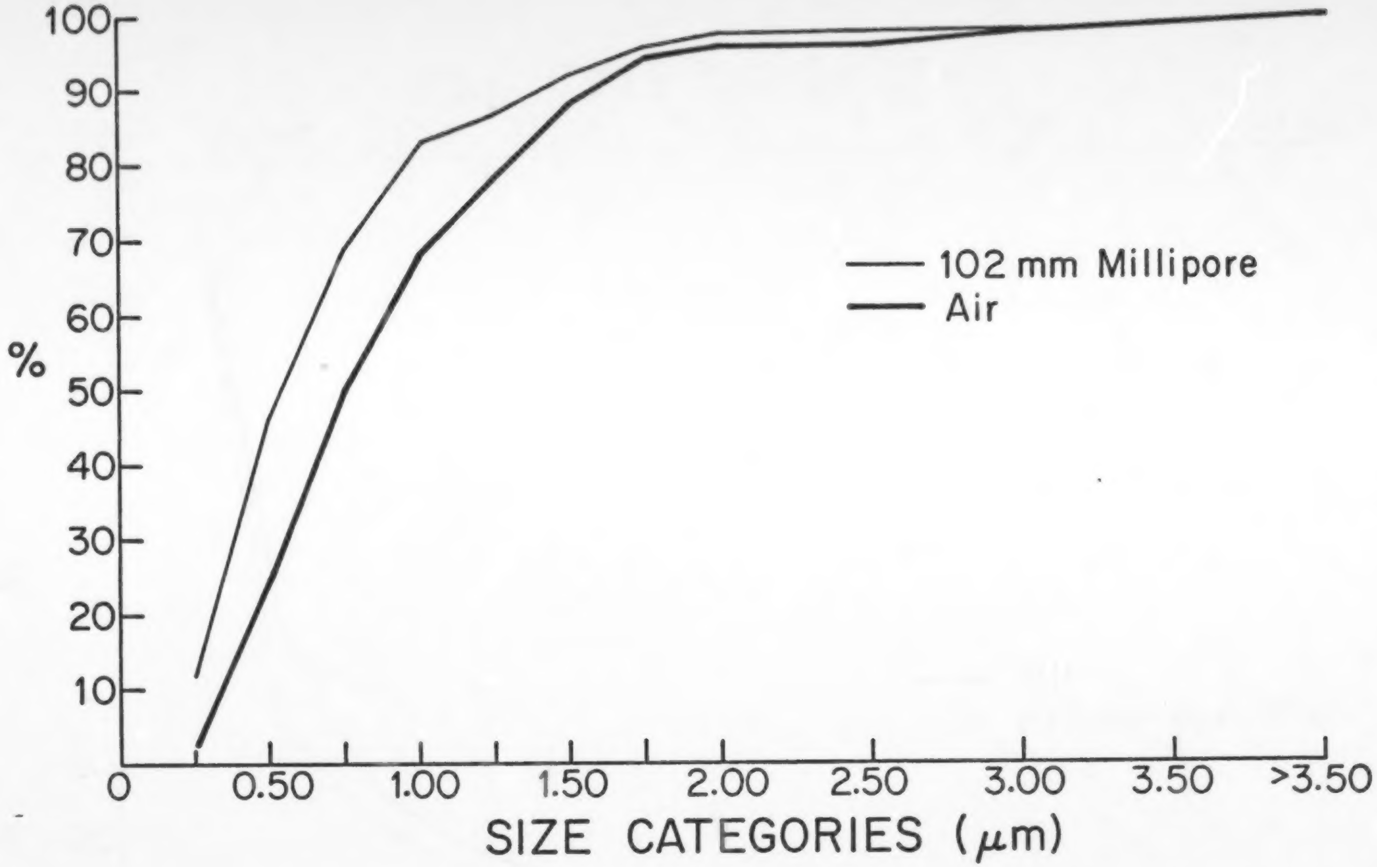

Figure 3. Cumulative percentage distributions for chrysotile lengths in air samples and blank Millipores.

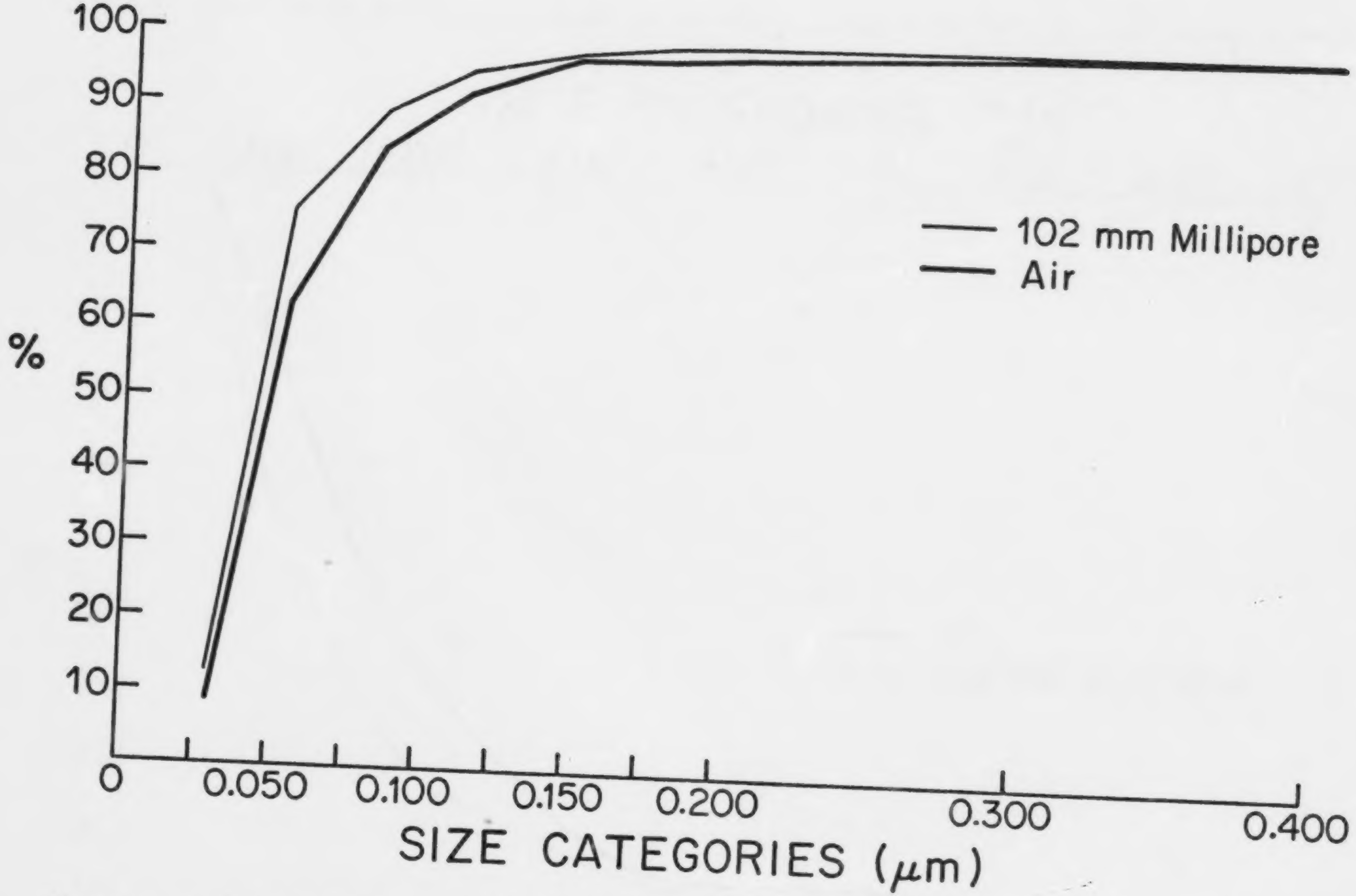

Figure 4. Cumulative percentage distributions for chrysotile widths in air samples and blank Millipores.

Table 7. Kolmogorov-Smirnov Test Results from the Comparison of the 102 mm Diameter Millipore and Metropolitan Air Sample Cumulative Percentage Distributions, Along with the $\bar{x}$ and x Values of the Fiber Widths and Lengths.

Comparison	Dimension	x^2	Conclusion (0.01 Level)
102 mm Millipore/Air	Width	8.78	Reject H_o
102 mm Millipore/Air	Length	21.94	Reject H_o

Samples	$\bar{x}$ Width (μm)	x Width (μm)	$\bar{x}$ Length (μm)	x Length (μm)
102 mm Millipore	0.053	0.042	0.755	0.550
Metropolitan Air	0.064	0.050	0.965	0.790

An estimate of the blank contribution to the chrysotile concentrations on the air samples was undertaken. Using the median Nuclepore and 102 mm diameter Millipore fibers per square millimeter values on the original filters (from Table 2) and the sample areas of the filters, the median total chrysotile fiber number on each filter component of the blanks and on the ashed air samples was calculated. Table 8 is a list of the total fiber values and the calculated chrysotile percentage contributions of the Nuclepore (including ashed dish) and Millipore filters to the air samples. Figure 5 is a graphic presentation of the data in Table 8. The ashing dish contribution to the Nuclepore was about 70 percent of the fibers on the Nuclepore.

Table 8. Values for the Percentage Contributions of the Millipore and Ashed Dish Nuclepore Filters to the Metropolitan Air Sample.

Sample area of Nuclepore = 960 mm^2

Sample area of .8,102 mm diameter Millipore = 6650 mm^2

Median Fibers in whole sample area of Nuclepore (+ Dish) = 132,480

Median Fibers in whole sample are of Millipore (including a correction factor of 1.23 for the no sample margin) = 1,341,438

Median Fibers in whole sample area of air sample = 9,110,500

Hypothetical Percent of Air Filter Ashed	Percent of Fibers From Millipore	Percent of Fibers From Nuclepore + Dish
100	13.2	1.4
90	13.2	1.6
80	13.2	1.8
70	13.2	2.1
60	13.1	2.4
50	13.1	2.9
40	13.0	3.5
30	12.8	4.7
20	12.5	6.9
10	11.7	12.8

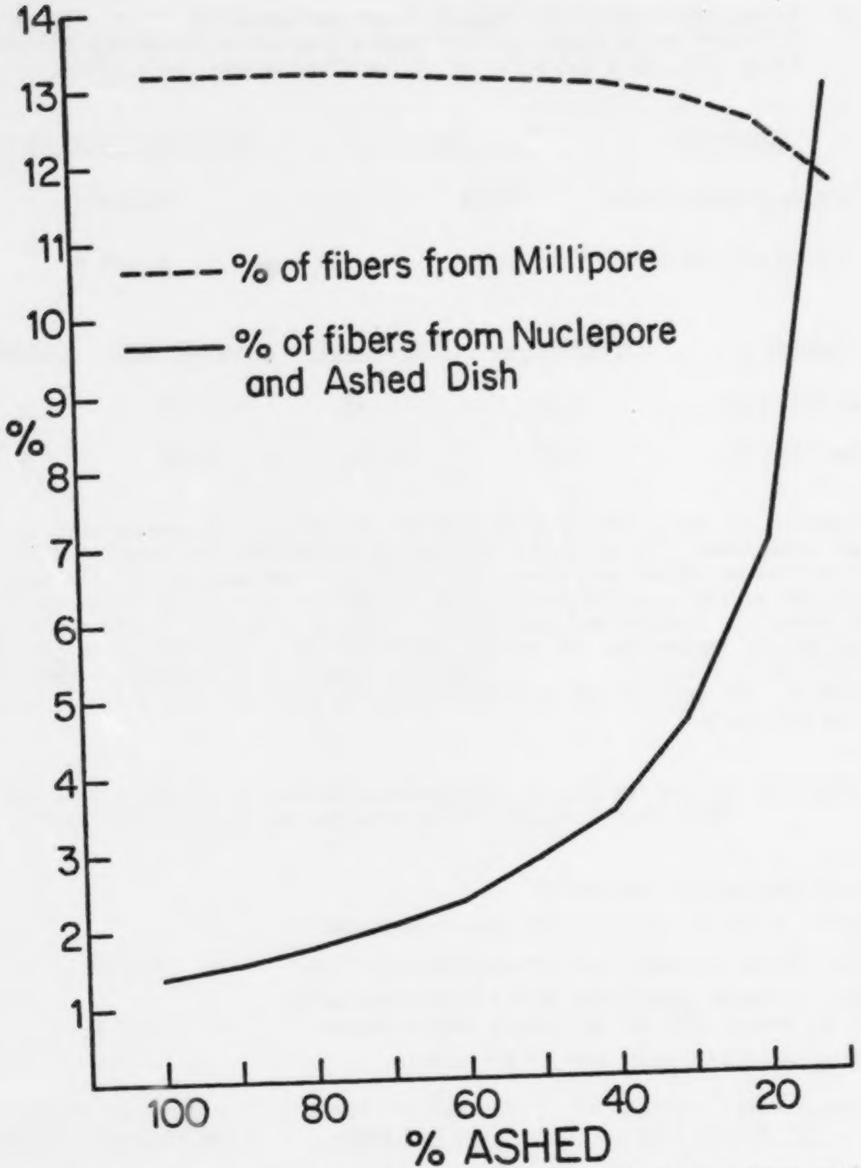

Figure 5. Percentage contributions of the Millipore and Nuclepore (with ashing dish) blanks to the sample chrysotile levels in a metropolitan air sample.

The blank contribution of chrysotile fibers to the sample chrysotile counts can be substantial, especially when small sections of a sample are ashed. As can be seen in Table 8 and figure 5, the relative contribution of fibers by the Millipore filter remains nearly constant. The Nuclepore-ashing dish contribution, on the other hand, becomes greater as the size of the filter ashed decreases. The size of the Nuclepore filter is independent of sample area ashed. The percentage values presented apply only to these samples. If the sample load were less, the blank contribution to the chrysotile fiber count would be proportionately greater.

Although additional statistical tests might be used to bolster the case, the fact that the size distributions as well as the numbers of fibers on these samples differ substantially leads one to conclude that each step in this preparation procedure adds contamination to the final analysis. In this study, we did not separate those blank samples which were ashed alone in the ashing chamber from those which were ashed with samples. Thus, there is the possibility that some cross contamination occurred within the ashing chamber on some of these samples. Our purpose was to evaluate what was actually occurring during the preparation of air samples. This preparation consisted of ashing two or three air samples along with an empty dish and Millipore blank in one ashing chamber. If contamination occurred, it was a part of the samples as well as the blanks.

It is also evident, at least in this study, that large variability in the blank concentrations makes comparison of a particular sample with the blank that was ashed with it an unsuitable technique for assessing contamination in the sample. The large variability will cause the contamination level to be frequently over- or underestimated. Instead, what is needed is overall statistical control, where the blank level and its variability are known. From this, one can determine when a particular preparation is out of control, and whether a particular sample concentration is significant. We suggest that the provisional method provide a thorough discussion of the contamination problem as well as sample preparation and counting errors so that when a sample is reported it will include confidence intervals for the fiber concentrations and an indication of whether that concentration is significantly above contamination levels.

2. Fiber Classification

In addition to the blank problem, another problem which may seriously affect the quality of the analyses is the fiber classification procedure. Except for the problems outlined by Millette [3] and Chatfield [4], the identification of chrysotile asbestos is easily accomplished using standard diffraction patterns. Chrysotile is scroll-like in structure and gives the same diffraction pattern regardless of how it is rotated about the fiber axis. For that reason, there is no problem with orientation when using electron diffraction to identify the mineral.

The situation for amphibole minerals is much different, and confusion in identification can result for many reasons. The provisional method provides four criteria to classify a fiber as amphibole. These criteria are that it must have a diffraction pattern with closely spaced spots arranged in rows, called layer lines, the spacing between the rows should represent a distance of about 0.52 nm, the pattern should look like those obtained from standards or published in the literature and its morphology should look like an amphibole.

Because amphiboles are not tubular in structure and are usually single crystals at the sizes which produce patterns in a 100KV TEM, different diffraction patterns are obtained with different orientations (rotations) about the fiber axis. Because of this, many amphibole fibers give patterns which may be atypical of patterns obtained from standards or those published in the literature. Misidentification can be the result. Figure 6 provides four amphibole patterns which are similar, but differ because of orientation.

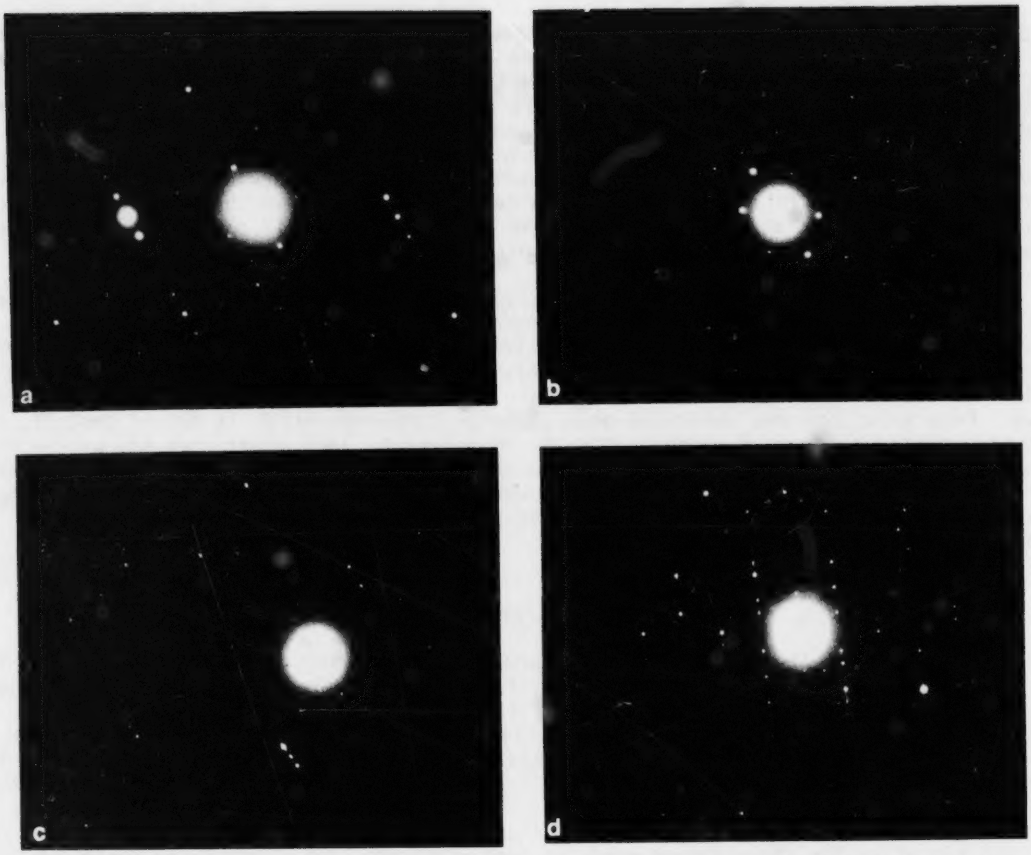

Figure 6. Four "typical" amphibole patterns (grunerite). Closely spaced spots form rows approximately 0.52 nm apart. Each pattern is different because different planes diffract when the fiber orientation to the electron beam is changed. a) near [301] zone axis, fiber has (100) plane almost perpendicular to electron beam b) near [312] zone axis fiber has (110) plane almost perpendicular to beam c) arcs of bright spot are Laue zones d) some streaking evident.

An additional problem to incorrectly classifying fibers which are in fact amphibole, is the possible identification of other minerals as amphibole. There are several minerals which give diffraction patterns which closely or superficially resemble those obtained from amphibole. Palygorskite, sepiolite, enstatite, hypersthene, laumontite, magnetite, and minnesotaite all give diffraction patterns which could be mistaken for amphiboles. This problem is further exacerbated by the fact that it is difficult or impossible to estimate distances in the darkness of an electron diffraction pattern on the TEM screen. Figure 7 shows six electron diffraction patterns which look like amphibole patterns. Plate a is an amphibole pattern. How easily can it be distinguished from the others? Thus, the provisional method is only adequate when the mineralogy of a sample is well known or when minerals with amphibole-like patterns are not present.

Unfortunately, uncomplicated or well characterized sources are rare. Increasingly, the method is being used to measure ambient air concentrations when there is no defined source of asbestos. Broad mixtures of mineral fibers may be found in rural or urban air or associated with mineral assemblages in mining operations. A laboratory which relies on the provisional method may find it extremely difficult to classify the fibers accurately. Mistakes are often very expensive and time consuming to correct and can lead to the loss of a laboratory's reputation.

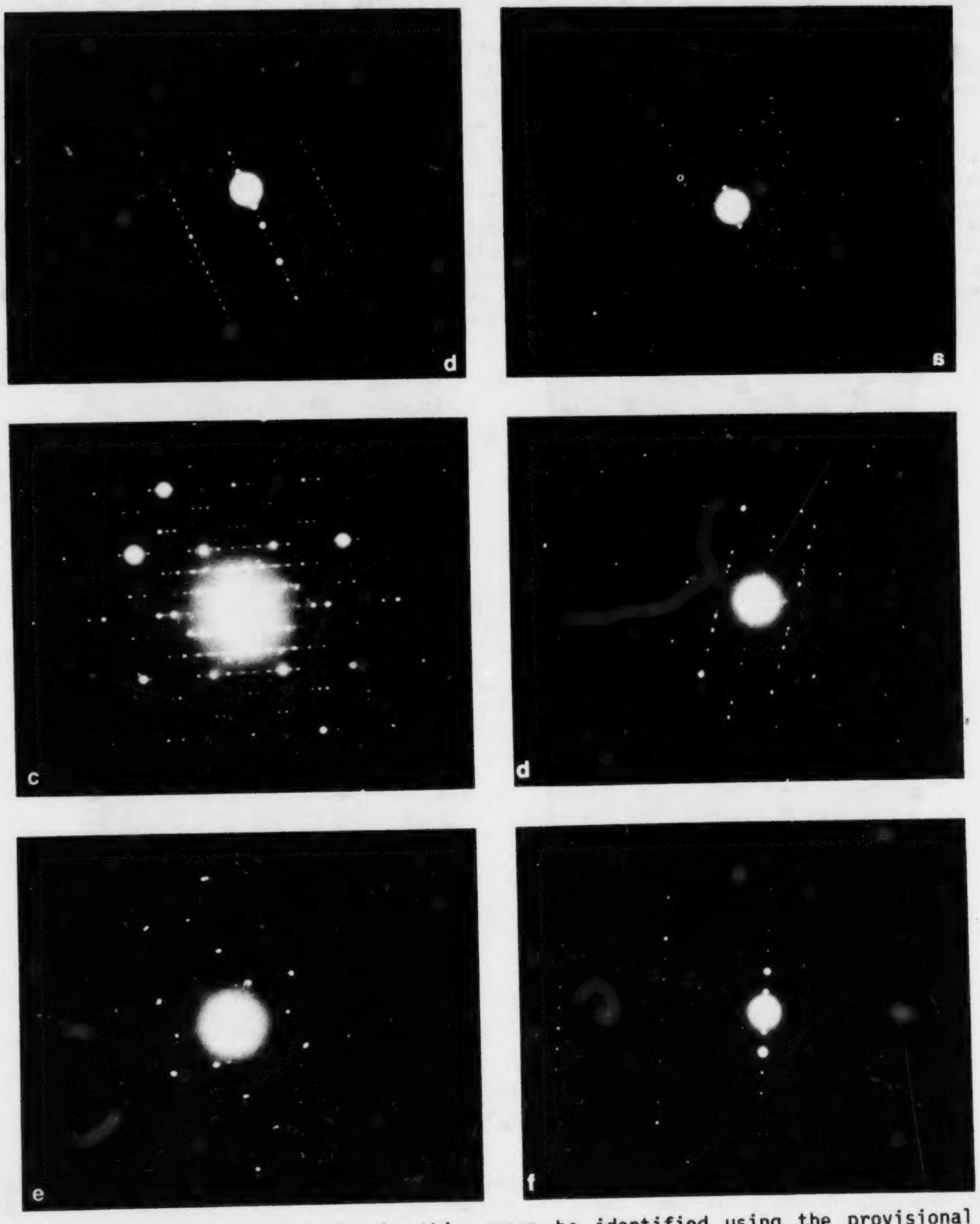

Figure 7. Can the amphibole in this group be identified using the provisional method? a) grunerite (amphibole) b) hypersthene c) minnesotaite d) sepiolite e) palygorskite f) laumontite.

There are several steps which could be taken which greatly increase the power of the method to separate the amphiboles from other minerals. As a first step, some means of accurately measuring the spacing between the layer lines in diffraction patterns should be implemented. This could be done by evaporating a thin gold film onto the grid to provide an internal diffraction standard or by requiring that all supposed amphibole diffraction patterns be photographed and the camera constant recorded and used to determine the actual spacing between layer lines. A gold film of proper thickness will not obscure detail in the image while providing the ring pattern which can be used as a ruler in the microscope. Although there is some variability in the spacing between layer lines in amphiboles, some interfering minerals could quickly be eliminated by this technique.

The use of energy dispersive spectroscopy (EDS) in conjunction with the measurement of the layer line spacing would greatly improve the capability of the analyst to distinguish amphiboles from other interfering minerals. Several laboratories are already using EDS on transmission or scanning microscopes. Making its use part of the standard method would greatly enhance asbestos identification capabilities. Unfortunately, there are still several minerals which might be incorrectly identified as amphibole based on EDS and layer line spacing. Figure 8 shows four EDS spectra corresponding to plates a, b, c, and d in figure 7. If the composition variability of amphibole minerals is considered (solid solution series), it is clear that it would be difficult distinguishing these fibers by EDS.

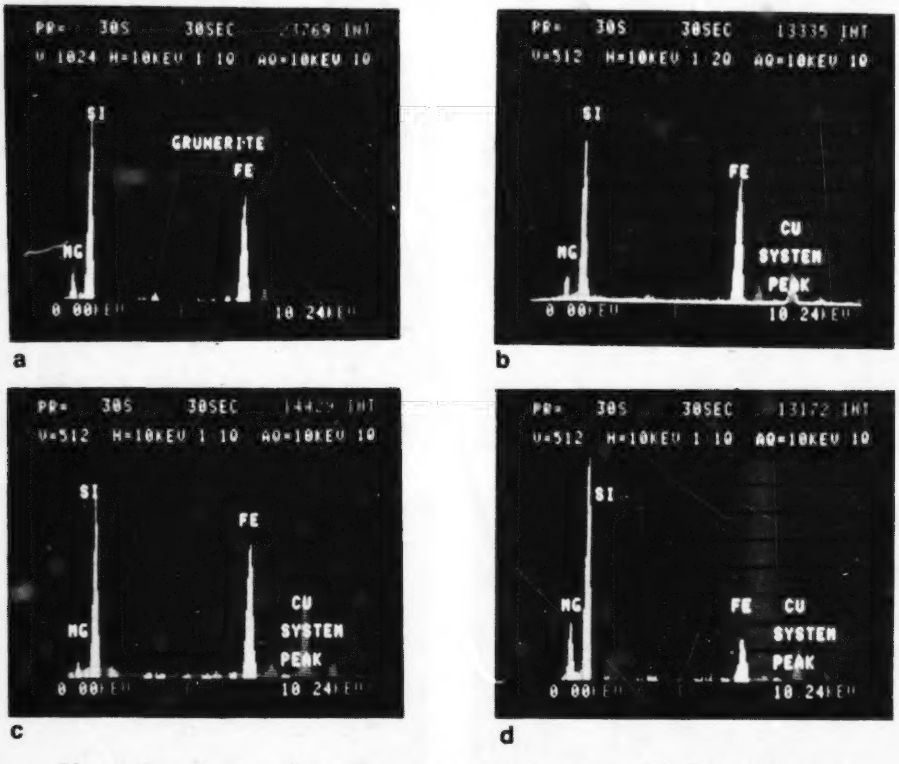

Figure 8. Energy dispersive x-ray spectroscopy (EDS) spectra from a, b, c, and d of figure 7.

In order to thoroughly differentiate these minerals from amphiboles, it is necessary to go one step further. If the fibers are tilted in the electron microscope so that the electron beam is parallel to a set of planes in the crystal (on a zone axis), the resulting diffraction pattern can be indexed. That is, the planes which produce each spot in the diffraction pattern can be identified. Patterns can be indexed as illustrated in figure 9 using the computer [5], using common zone axis electron diffraction patterns [6], or by hand with tables. Used in conjunction with EDS or along with more than one pattern, this will provide positive identification of the fibers and evidence which can be verified by someone else.

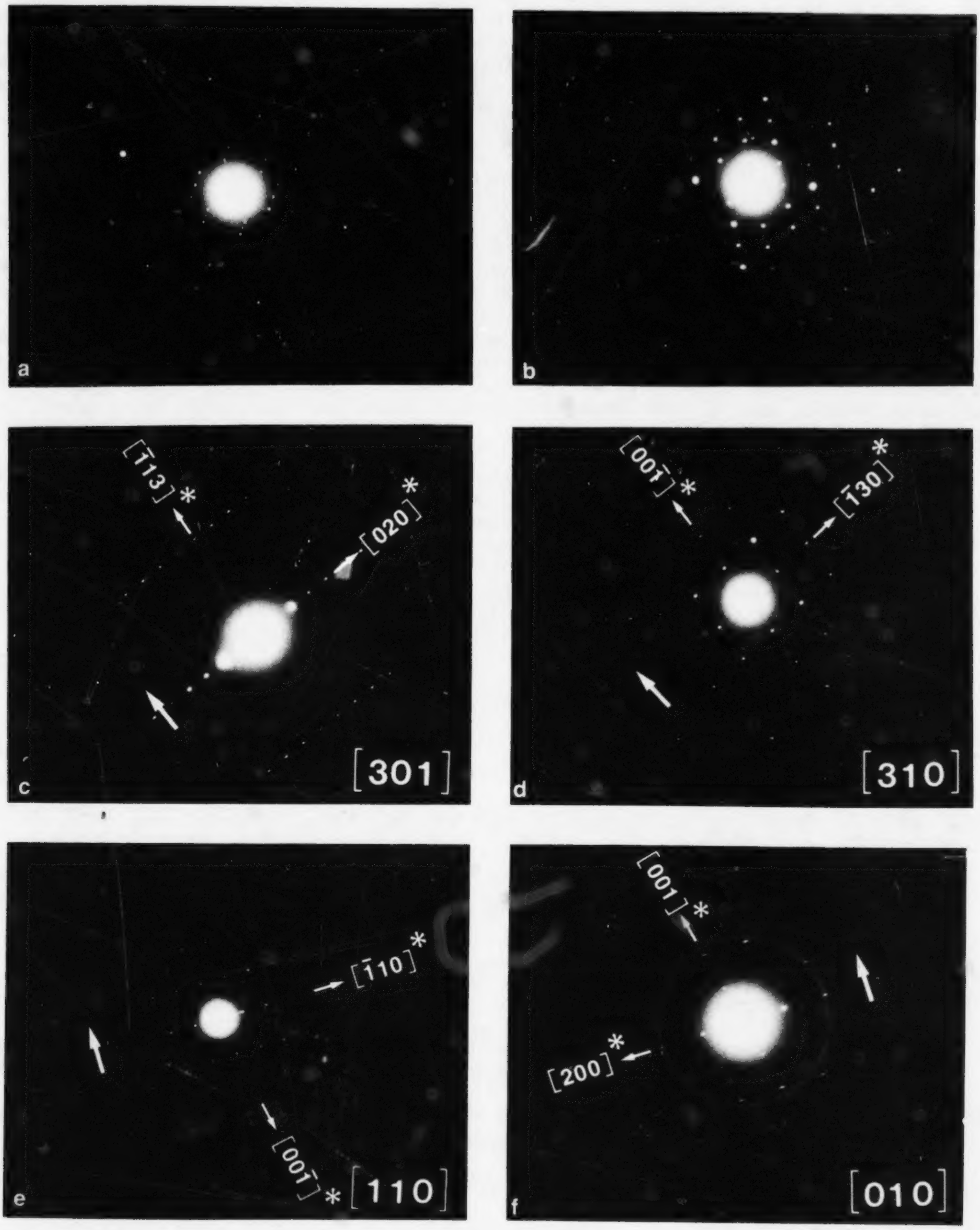
a
b
[1̄13]*
[020]*
c
[301]
[001̄]*
[1̄30]*
d
[310]
[1̄10]*
[001̄]*
e
[110]
[001]*
[200]*
f
[010]

Figure 9. Zone axis patterns. Bright ring is from gold film (111) plane and represents a d value of 0.2355 nm. Gold ring is sufficient to exclude a and b from amphibole category (minnesotaite and laumontite). c, d, e, and f show four common amphibole zone axes. Large arrow gives the orientation of the fiber to the pattern.

It may be argued that tilting and indexing patterns is too time comsuming and not suitable for routine work and that it should only be used when needed. Unfortunately, one does not know when there are interfering minerals present unless rigorous techniques are used to identify them. It must first be determined that interfering minerals are not present then less rigorous and less time consuming methods can be used.

3. Conclusions

The provisional method should provide an adequate method of addressing the contamination levels associated with these analyses. Our experience with the blank problem provides evidence that contamination is found at each stage of the sample preparation procedure. Although this analysis pertains mostly to the procedure which uses low temperature ashing, there clearly is some contamination on the Nuclepore filters themselves. This might be important in lightly loaded samples. We suggest that the effective blank levels be reported along with fiber concentrations and confidence intervals for the fiber count. Ultimately, some expression of the blank variability should be reported. Thus, as suggested in the provisional method, the minimum detection limits might be the blank level plus three standard deviations of that level.

Finally, the method should provide for the rigorous classification of the fibers using zone axis diffraction patterns and EDS. It may not be necessary to index every pattern obtained from every fiber in a sample, but some part of the standard method should provide that when a sample with an unknown assemblage of minerals is brought into a lab some percentage of the fibers, and particularly those identified as amphibole, should be examined by EDS and quantitative electron diffraction. The techniques should become a part of the capabilities of any lab analyzing asbestos by electron microscopy. If a laboratory can show that no interfering minerals are present, then certainly less rigorous methods may be used.

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MSHA STANDARD METHOD FOR FIBER IDENTIFCATION B ELECTRON MICROSCOPY

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ABSTRACT

The EPA Provisional Methodology Manual represents an eclectic compendium of techniques employed in the analysis of airborne particulates for asbestos by electron microscopy. Philosophically, the methods tested and the resulting conclusions have a certain degree of universal applicability. In practice, however, absolute adherence to the method may not be possible due to differences in mandated standards, methods, and techniques.

The Mine Safety and Health Administration has developed a standard method for the analysis of airborne particulates which roughly parallels the EPA method. Variations in the technique include optical microscopy for fiber count, transmission electron microscopy (TEM) for photomicroscopy and selected area electron diffraction (SAED), scanning transmission electron microscopy (STEM) for image analysis, and energy dispersive x-ray spectrometry (EDS) for elemental composition.

Applications of the technique as a routine analytical method will be discussed, with particular emphasis on areas of variation from the EPA method.

Key Words: Electron microscope; energy dispersive x-ray spectrometry; image analysis; scanning transmission; selected area electron diffraction; transmission electron microscope.

1. Introduction

From the outset, it may be advantageous to state basic agreement with what I perceive to be the pervading philosophy contained in the EPA Provisional Methodology. That is, the application of minimal sample manipulation which yields a viable analysis reflective of actual environmental conditions.

However, each phase in the construction of the Provisional Methodology was predicated upon absolute latitude in the selection process, such that the entire analytical procedure has an optimized quality. This degree of freedom in the selection process is not always available, especially at the analytical level, where specific definitions and mandated techniques demand a different approach to the solution of similar problems.

What will be described in this paper is an independently developed method which generally parallels the Provisional Methodology, but evolved in response to the imposition of somewhat different constraints.

2. Overview of the MSHA Method

The methods employed by the Mine Safety and Health Administration in the analysis of airborne particulate samples for asbestos is governed by specifications found in the Code of Federal Regulations [1][1]. Therein, the term "fiber" is defined as any particulate with a three to one or greater length to width aspect ratio, and a length of five micrometers or longer. The term "asbestos" is recognized as generic, applicable to a number of hydrated silicates, but its use is specifically limited to describe the minerals chrysotile, amosite, crocidolite, anthophylite asbestos, tremolite asbestos, and actinolite asbestos.

An optical microscopy method, generally known as phase contrast [2], is also specified for use in obtaining gross fiber counts for each sample filter. It is assumed that the entire fiberous content of the sample, as preceived by the optical microscopy method, has the potential of classification as asbestos. Therefore, if the fiber count established by optical microscopy exceeds the threshold limit value (TLV) for asbestos [3], the sample must also be examined by electron microscopy for fiber identification.

Electron microscopy is utilized to obtain a physical characterization of the analyte material, and to make an analytical determination of the nature of specific components of the analyte. Physical characterization is made by a computer assisted image analysis system. Analysis of the analyte is achieved by the classification of individual fibers based upon the crystallographic data contained in selected area electron diffraction (SAED) patterns in combination with elemental compositional data derived from energy dispersive x-ray spectrometry (EDS).

Results from the optical and electron microscopy methods are combined to yield the number of asbestos fibers per unit of air. These data are used to establish if a health problem exists in a specific mining operation, and if so, the nature and extent of that problem.

3. Specimen Preparation

The first variation from the EPA Provisional Methodology, as well as a number of subsequent variations, was necessitated by the use of the phase contrast optical microscopy method. This method specified the use of cellulose acetate membrane filters for sample collection instead of the polycarbonate filters recommended in the Provisional Methodology. In turn, the use of cellulose acetate filters precludes an initial carbon deposition, since replication of surface features would obscure the analyte. However, this same quality of surface roughness acts as an effective entrainment media, thereby minimizing analyte losses that would be incurred during subsequent transportation from remote sampling locations.

Specimens of each sample are prepared on 10 nm thick carbon substrates. The carbon substrates are produced by floating a thin film of one percent parlodion (high purity cellulose nitrate) dissolved in amyl acetate on the surface of reagent grade water. Electron microscope grids are laid on the parlodion film, and the film covered grids are removed to the vacuum evaporator for carbon deposition. The parlodion film acts only as a temporary support for the deposition of carbon, and thereafter is removed by dissolution in acetone vapor using a modified Jaffe washer [4].

Two sections of the filter are excised adjacent to, and on opposite side from the wedge removed for optical microscopy. These are laid, filtrate side down, on individual carbon substrate covered electron microscope grids contained in a Jaffe washer. After about twenty-four hours exposure to acetone vapor, the cellulose acetate filter is completely dissolved, and each specimen receives a second carbon coating which effectively sandwiches the analyte between two layers of carbon.

All substrate and specimen preparation, exclusive of carbon deposition, is performed in separate class 100 environmental chambers, both for the safety of personnel involved and to prevent specimen contamination. Sheets of clean room quality polyurethane are used in preference to fiberous filter papers in the Jaffe washer, and for all cleaning application within the environmental chambers. Blank specimens are prepared in conjunction with each group of specimens to test the integrity of the system.

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[1] Figures in brackets refer to the literature references at the end of this paper.

A number of grid materials and mesh sizes have been explored for applicability to analytical electron microscopy, carbon coated composites, beryllium, and gold in size ranges from 75 to 400 mesh. The carbon composite grids proved unusable due to an inherent dimensional instability which caused them to curl during substrate preparation. Both beryllium and gold grids have been successfully applied in the production and analysis of asbestos mineral specimens. However, the openings of the beryllium grids are poorly defined; they are difficult and expensive to obtain in large quantities, and their use poses some very significant health hazards. For these reasons, gold grids appear to be the best selection for large scale routine asbestos analysis.

With respect to grid mesh size, grids with large openings provide a significantly higher percentage of open area for analysis. An example can be found by contrasting a 100 mesh grid with a 400 mesh grid, where the 100 mesh grid will have nearly twice as much open area. The increase in open area, however, means a loss in specimen support, with a resulting increase in specimen substrate failure. Also, each grid opening contains more than one field of view, and therefore care must be exercised that a small degree of overlap is maintained in each field of view as a grid opening is examined. These disadvantages notwithstanding, the increased efficiency realized by the use of large mesh size grids makes their selection advisible.

4. Analytical Procedures

Specimens of the sample are systematically examined in a scanning transmission electron microscope (STEM) at an acceleration voltage of 100 keV and a magnification of 3700 diameters. Each fiber encountered during the examination is photographed at 6000 diameters, and an SAED pattern is obtained and photographically recorded at zero degree tilt angle.

The STEM unit is then switched to the scanning mode at 20 keV, and computer aided image analysis is utilized to obtain individual fiber measurements and statistical data relevant to those measurements. The accumulated statistical data generated by the image analysis system, and printed at the end of each field of view, is used to determine when adequate data for the physical characterization of the analyte has been achieved.

A deadtime corrected EDS spectra is accumulated for 200 seconds from each fiber at a rate of 300 counts per second. A computer program compares this spectra to an internally stored linear regression and quadratic plots of intensity versus concentration for 10 key elements (Na, Mg, Al, Si, K, Ca, Ti, Cr, Mn, and Fe), and calculates elemental composition for the unknown spectra. The internal plots utilized in this calculation were generated by actual analysis of chrysotile and the five specified asbestiform amphibole minerals obtained from at least two different sources, and represented by at least 10 different spectra from each mineral species.

At the end of analysis all SAED patterns are intensely examined, and each fiber is classified as either chrysotile, asbestiform amphibole, not asbestos, or ambiguous. The classification of an SAED pattern produced by chrysotile is relatively straight forward, since these patterns are composed of uniquely streaked diffraction spots arranged in well defined arrays known as layer lines, which approximately correspond to a 0.53 nm periodicity [5,6]. Other minerals can also exhibit a 0.53 nm layer line periodicity; however, none of these display the prominent streaking so evident in the case of chrysotile.

Differentiation between asbestiform amphibole minerals and associated nonasbestos minerals can also be made by careful and critical inspection of the SAED pattern. The characteristic patterns produced by the asbestiform amphibole minerals will have prominent layer lines, numerous reasonably well defined diffraction spots that are closely spaced on each layer line, a 0.53 nm layer line periodicity, and low angle diffraction spots. However, individual members of the mineral group cannot be readily distinguished by visual analysis of the SAED pattern.

Species identification of the asbestiform amphibole minerals is achieved by confirmation that the SAED pattern is typical of this group, and that the computed elemental composition derived from the EDS spectra is within the published compositional range [7-11] of one of these minerals for all 10 key elements. If either of these two identification criterion of positive SAED pattern classification as asbestiform amphibole and a fit of the EDS spectra to the published compositional data are not achieved, then the fiber cannot be identified as asbestos.

Fibers which produce SAED patterns that cannot be positively classified as chrysotile or asbestiform amphibole, are classified as not asbestos. Typically, such an SAED pattern would fail to have a definite layer line morphology (i.e., quasi-hexagonal array), a layer line periodicity not equal to 0.53 nm, an inadequate number of diffraction spots, or a combination of the above conditions. Fibers which do not yield SAED patterns, or which produce patterns with insufficient chrystallographic data are classified as ambiguous.

5. Summary

The methods outlined in this paper are the result of both practical experience and carefully designed experimentation. It is neither the intent nor the essence of this presentation to advocate a system, method, or technique simply for the purpose of "marching to the beat of a different drummer".

Instead, what has been described is a viable analytical method evolved in response to a specific set of circumstances other than those imposed in the development of the EPA Provisional Methodology. Although this violates, so to speak, the letter of the law, the spirit remains intact.

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